VOL. IX, No. 4 JULY 1958

PLANT AND SOIL

International Journal of Plant Nutrition, Plant Chemistry, Soil Microbiology and Soil-borne Plant Diseases

UNIVERSITY OF HAWAII
LIBRARY
SEP 23 '58

MARTINUS NIJHOFF — PUBLISHER — THE HAGUE

PLANT AND SOIL

International Journal of Plant Nutrition, Plant Chemistry, Soil Microbiology and Soil-borne Plant Diseases

Revue internationale de nutrition des plantes, de chimie végétale, de microbiologie du sol et des maladies des plantes en rapport avec le sol

Internationale Zeitschrift für Pflanzenernährung, Pflanzenchemie, Bodenmikrobiologie und bodenbedingte Pflanzenkrankheiten

Issued under the auspices of the "Koninklijk Genootschap voor Landbouwwetenschap" (Royal Netherlands Society of Agricultural Science)

Executive Editors: D. I. Arnon, Berkeley; J. Baeyens, Louvain; F. C. Gerretsen, Groningen; G. W. Harmsen, Groningen; E. G. Mulder, Wageningen; F. J. Richards, London; A. C. Schuffelen, Wageningen; F. Steenbjerg, Copenhagen. **Secretary:** G. H. Arnold, Groningen.

Consulting Editors: Wm. A. Albrecht, Columbia, Mo.; C. Arnaudi, Milan; E. Gäumann, Zurich; L. Gisiger, Liebefeld-Berne; F. G. Gregory, London; A. J. de Groot, Groningen; J. den Holder, Ghent; T. H. van den Honert, Leyden; V. J. Koningsberger, Utrecht; J. Lavollay, Paris; W. J. Lütjeharms, Bloemfontein; H. Lundegårdh, Uppsala; E. Melin, Uppsala; M. Mes, Pretoria; M. Ødelien, Ås; E. J. Petersen, Copenhagen; C. S. Piper, Adelaide; H. G. Thornton; Harpenden, Herts.; S. Tovborg Jensen, Copenhagen; A. I. Virtanen, Helsinki; S. A. Waksman, New Brunswick; F. W. Went, Pasadena; Joh^{a}. Westerdijk, Baarn; J. G. Wood, Adelaide.

CONTENTS: TABLE DES MATIÈRES: INHALT:

C. D. Sutton and *E. G. Hallsworth*, Studies on the nutrition of forage legumes. I. The toxicity of low pH and high manganese supply to lucerne, as affected by climatic factors and calcium supply 305

K. C. Lu and *W. B. Bollen*, Effect of Gibrel, a potassium salt of gibberellic acid, on microbial activities in soil 318

S. M. Bromfield, The properties of a biologically formed manganese oxide, its availability to oats and its solution by root washings 325

P. H. Nye and *W. N. M. Foster*, A study of the mechanism of soil-phosphate uptake in relation to plant species 338

T. W. Walker and *A. F. R. Adams*, Competition for sulphur in a grass-clover association 353

Paul Simonart et Jaques Mayaudon, Étude de la décomposition de la matière organique dans le sol, au moyen de carbone radioactif. I. Cinétique de l'oxydation en CO_2 de divers substrats radioactifs 367

Jaques Mayaudon et *Paul Simonart*, Étude de la décomposition de la matière organique dans le sol au moyen de carbone radioactif. II. Décomposition du glucose radioactif dans le sol. A. Répartition de la radioactivité dans les fractions humiques du sol 376

Paul Simonart et *Jacques Mayaudon*, Étude de la décomposition de la matière organique dans le sol au moyen de carbone radioactif. II. Décomposition du glucose radioactif dans le sol. B. Répartition de la radioactivité dans l'α-humus 381

J. M. Wybenga and *J. J. Lehr*, Exploratory pot experiments on sensitiveness of different crops to sodium: E. Red table beet 385

Printed in the Netherlands

Plant and Soil IX, 305—394 The Hague July 1958

Plant and Soil IX, no 4 July 1958

STUDIES ON THE NUTRITION OF FORAGE LEGUMES

I. THE TOXICITY OF LOW pH AND HIGH MANGANESE SUPPLY TO LUCERNE, AS AFFECTED BY CLIMATIC FACTORS AND CALCIUM SUPPLY*

by C. D. SUTTON and E. G. HALLSWORTH

University of Nottingham, School of Agriculture

INTRODUCTION

As part of a programme designed to investigate the effects of acid soils on the growth of several forage legumes, the behaviour of lucerne (*Medicago sativa*) variety Du Puits, and white clover (*Trifolium repens*) variety S. 100 was studied, when grown in both agar and water culture. From the factors which Hewitt [5] suggested to exist in the acidity complex, those of hydrogen-ion concentration, calcium, manganese and aluminium supply were chosen for study in factorial experiments. Lucerne was found more sensitive to all the chosen factors, and the results reported in this paper refer to the effects of a low pH, and a high manganese level on this species only. Initial results of experiments conducted in a glasshouse suggested that climatic factors were concerned in the severity of manganese toxicity. Similar findings had been reported by McCool[8] and Lohnis [7] and an experiment was therefore conducted to to investigate the influence of temperature and light intensity under controlled conditions.

EXPERIMENTAL METHODS

(a) *Agar culture*

In the agar culture, plants were grown aseptically on 25 ml of a 1% nutrient-agar slope in 6 × 1¼ inch test tubes, plugged with cotton wool.

* The data given here are taken from the thesis submitted by C. D. Sutton for the degree of Ph. D. of the University of Nottingham.

The nutrient solution used is given in Table I. One plant per tube was grown, and the seeds were surface sterilized, then inoculated with a pure strain of *Rhizobium meliloti* before being sown.

TABLE I

The nutrient solution used in the agar culture				
Element	Concentration (ppm)			Source
	Basic Components			
P	52			$Na_2C_3H_5(OH)_2PO_4.5\frac{1}{2}H_2O$
K	135			KCl
Mg	40			$MgSO_4.7H_2O$
Fe	6			Sequestrene complex
B	0.5			H_3BO_3
Mo	0.1			$(NH_4)_6Mo_7O_{24}.4H_2O$
Cu	0.1			$CuSO_4.5H_2O$
Zn	0.1			$ZnSO_4.7H_2O$
	Variable components			
N	0	14	140	NH_4NO_3
Ca*	15	60	300	$CaCl_2.6H_2O$
Mn	0.5	25	50	$MnSO_4.4H_2O$
pH	4	5	6	adjusted by means of H_2SO_4 or NaOH

* In some of the earlier experiments Ca levels of 27, 67 and 300 ppm were used, but results from these are not referred to here except in Table III.

(b) *Water culture*

The nutrient solution used in the water-culture experiments was similar to that given in Table I, except that the concentrations of P, K and Mg were reduced to 26, 60 and 20 ppm respectively. The plants were grown in 350-ml capacity jam-jars, painted black to exclude the light. One plant per jar was grown and the solutions were changed twice weekly during the first fortnight while the plants were very small, and thereafter three times per week. The pH was checked by means of a Pye portable pH-meter and the maximum shift recorded was $\pm$ 0.4 units.

(c) *Experimental layout*

For all experiments a balanced 3^4 design was used in a randomised block layout (9 blocks of 9 treatments), three factor interactions being confounded with blocks. Where only three factors were used, three replicates of each treatment allowed the use of the same statistical design. The majority of the experiments were conducted in a glasshouse, where heating was by convection heaters, and supplementary lighting, to give a 16-hour day length, was supplied by mercury-vapour lamps. One agar experiment (No. 24) was carried out in controlled-environment chambers where two light intensities and two temperatures could be applied The actual climatic conditions used were as follows: –

TABLE II

Climatic conditions in Experiment 24		
Block	Temperature (°C)	Light intensity* (Foot candles)
I	25	1,500 — 1,620
II	25	380 — 520
III	15	1,400 — 1,580
IV	15	350 — 500

* The light intensity was measured inside a plugged tube, the range indicating the extremes recorded over the whole block area.

The dry-weight yields of the plants were recorded at harvest (generally after six weeks growth, but in the control chambers after four weeks), and some chemical analyses were made of the plant material.

RESULTS

(a) *Toxicity of a high hydrogen-ion concentration*

It was found that the toxicity of the nutrient solution at pH 4 was not constant, but was affected by the level of some nutrients (particularly calcium and nitrogen); by the culture method employed, and by the environmental conditions.

Arnon and Johnson [2] concluded that over the pH range 4–8, hydrogen-ion concentration itself was not a toxic factor. The results of the agar experiments were in general agreement with this statement, as although some plants at pH 4 were extremely small and stunted, and showed symptoms of 'acid injury' (*i.e.* short swollen tap roots with small brownish laterals, which did not penetrate the agar), good growth could be obtained with a high supply of calcium and nitrogen. Fig. 1 which is taken from the results of a typical agar experiment shows the effects of increasing the nitrogen supply at the three pH levels. The plants at pH 4 receiving no added nitrogen [N(0)] showed toxicity symptoms, whereas those at the N(14) and N(140) levels were symptom free, with long slender white root systems. The total yield obtained, however, was much greater at pH 5 than at pH 4, and greater still at pH 6. Fig. 2 is a typical example of the increased growth which resulted from a higher calcium level at pH 4. Here an increase in the calcium level from 15 to 300 ppm completely removed the reduction in growth at the lower pH levels. Chemical analyses of the plant material showed that the uptake of calcium was generally lower at pH 4 than at pH 6, at all levels of calcium supply (Table III).

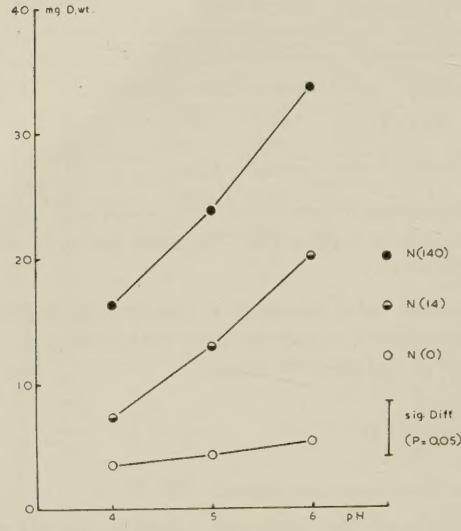


Fig. 1. The effect of increasing the nitrogen supply at the three pH levels, on the dry-weight yield of lucerne shoots in an agar-culture experiment.

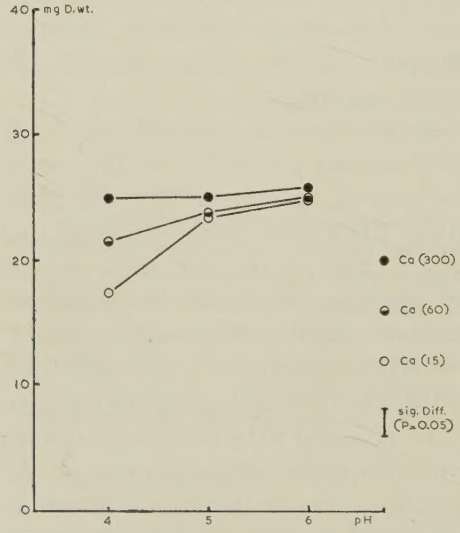


Fig. 2. The effect of increasing the calcium supply at the three pH levels on the yield of lucerne shoots in an agar-culture experiment.

TABLE III

Mean calcium content (as % of dry matter) of plants grown on agar at different pH levels (bulk sample of 5 replicates).

Calcium supplied (ppm)	27	67	300	Mean
pH 4	0.602	0.949	1.440	0.977
pH 5	0.668	1.244	1.778	1.230
pH 6	0.745	1.280	1.880	1.302
Mean	0.672	1.158	1.699	

In water culture, however, the nutrient solution at pH 4 showed a toxicity which was not relieved by the higher levels of calcium or nitrogen supply. (See Figs. 3 and 4). In this culture method a simi-

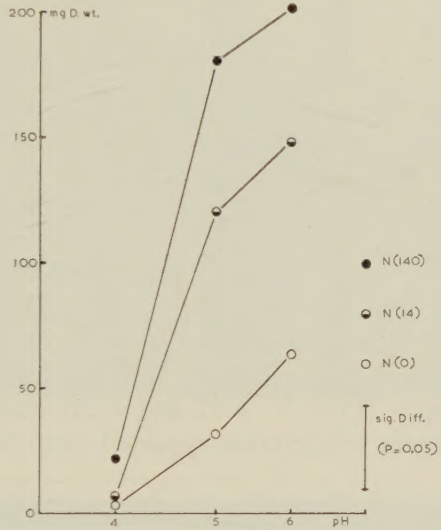


Fig. 3. The effect of increasing the nitrogen supply at the three pH levels on the yield of lucerne shoots in a water-culture experiment.

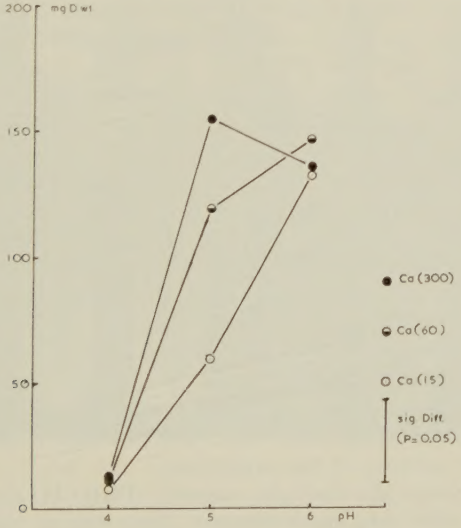


Fig. 4. The effect of increasing the calcium supply at the three pH levels on the yield of lucerne shoots in a water-culture experiment.

lar hydrogen-ion concentration appeared much more toxic than in agar culture. This aspect was investigated further using sand culture, with different-sized sand grains, and two rates of solution renewal. It was found that resistance to toxicity was associated with a pH change near to the plant roots. If this changed zone could be easily maintained, *i.e.* where the rate of solution renewal was slow, and the sand size was large, then the plants showed no injury at pH 4. If on the other hand the rate of renewal (in very fine sand or water culture, with frequent changing of the nutrient solution), prevented the formation of a changed zone, then the plants showed typical symptoms of injury.

These symptoms of acid injury were also affected in agar culture by variation in the environment (Experiment 24). It can be seen from Plates I–VIII that at the low temperature, plants at pH 4 had injured root systems, particularly at the high light intensity and low calcium supply, (Plate V, 4002 and 4022) whereas the plants at pH 4 at the higher temperature appeared quite normal, (Plate I,

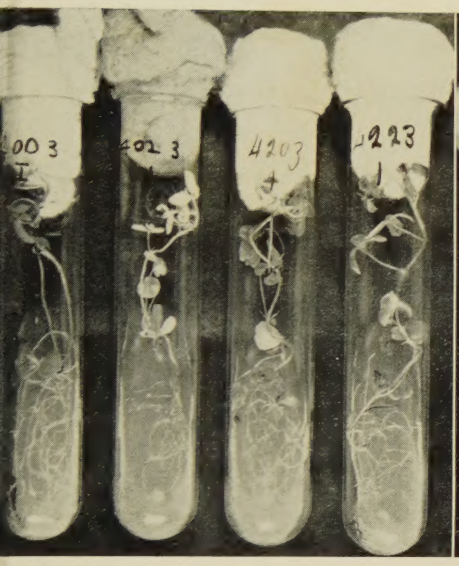
---

Plates I–IV: Plants grown at high temperature (25°C) and two light intensities. I and II receiving 1500–1620 ft. candles and III and IV receiving 380–520 ft. candles. *Manganese* supply: Nos. 4003, 4203, 6003, and 6203 receiving 0.5 ppm Mn; nos. 4023, 4223, 6023, and 6223 receiving 50 ppm Mn. *Calcium* supply: Nos. 4003, 4023, 6003, and 6023 receiving 15 ppm Ca; nos. 4203, 4223, 6203, and 6223 receiving 300 ppm Ca. *pH*: Nos. beginning with 4 – pH4; nos. beginning with 6 – pH 6.

With both high temperature and light intensity, the plants with a low manganese supply (4003, 4203, 6003, 6203) were large and healthy. At 25 ppm manganese, there were symptoms of excess manganese present, as light to dark brown irregular spots and blotches predominantly near to the tips of the leaflets. High calcium supply reduced the effect of these symptoms. At high manganese supply (50 ppm) the plants appeared stunted with severe blotching even at the high calcium levels (4223 and 6223) whilst a severe chlorosis can be seen at 15 ppm calcium (4023 and 6023). In the plants exhibiting these chlorotic symptoms all the leaflets were a uniformly bright yellow. The alleviating effect of the calcium can be seen by comparing 4023 with 4223, and 6023 with 6223.

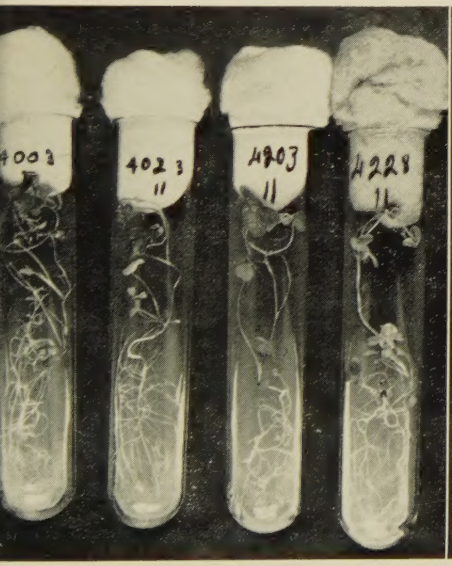
Whenever the shoots were exhibiting symptoms of manganese excess, the roots were reduced in size and were a golden yellow deepening to brown, depending on the severity of the symptoms.

At high temperature and low light intensity (Plates III and IV) the symptoms of toxicity were very much reduced. There was no chlorosis (compare 4023 – Plate III with 4023 Plate I), and symptoms of spotting occurred only in those plants receiving high manganese and at the two lowest calcium levels.



LATE I

PLATE II



LATE III

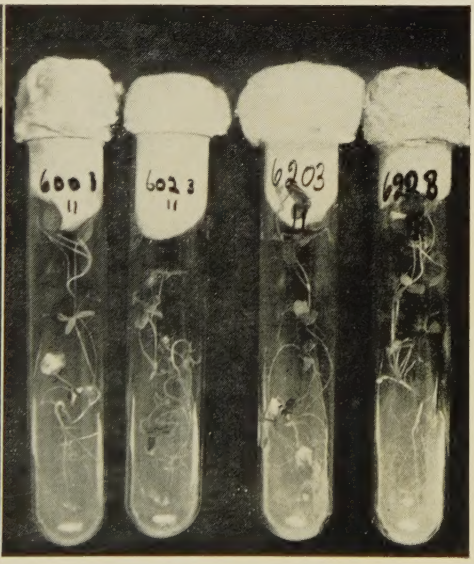


PLATE IV

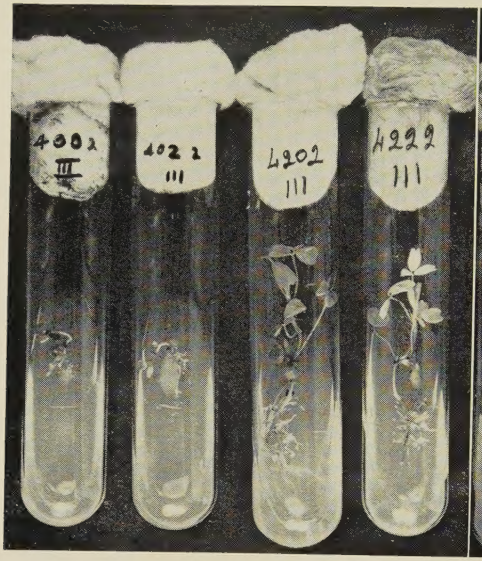


PLATE V

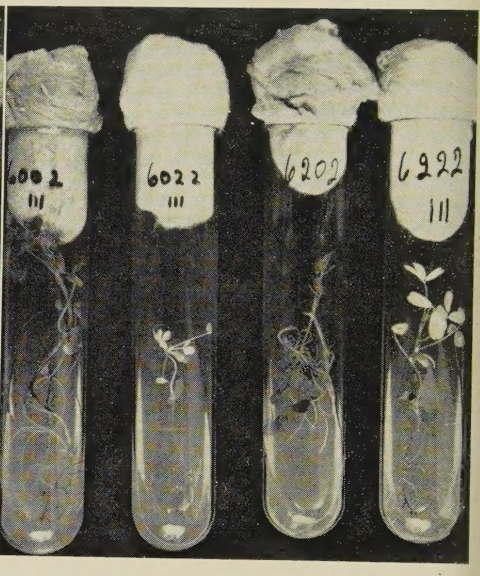


PLATE VI

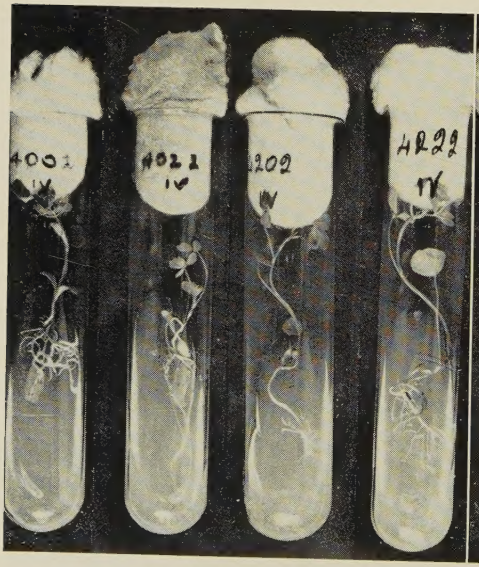


PLATE VII

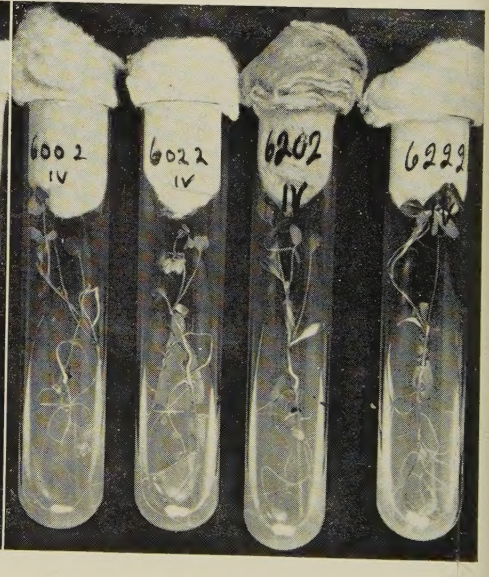


PLATE VIII

4003, 4023). When the yields of these plants were considered, however, the effect of light intensity was found to be more pronounced than that of temperature (Fig. 5). This shows that the

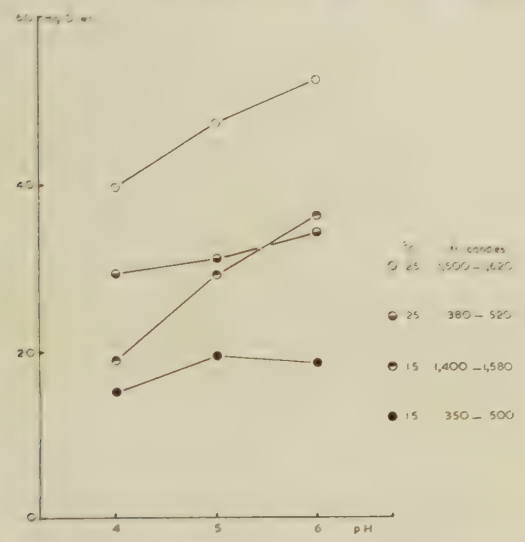


Fig. 5. The effect of the climatic treatments in Experiment 24 on the response of lucerne shoots at the three pH levels.

greatest difference between the pH levels was at higher light intensity, and it was only under these conditions that there was an interaction noted between pH and calcium. (See Figs. 6 and 7). The significance of this effect of light intensity upon the interaction

Plates V–VIII: Plants grown at low temperature (15° C) and two light intensities. Plates V and VI receiving 1400–1580 ft candles and VII and VIII receiving 350–500 ft. candles. Manganese and calcium supplies and pH's analogous to those of the plants of Plates I–IV.

With high light intensity and low temperature, growth was not quite so vigorous as at the higher temperature. The symptoms of excess manganese were the same as at high temperature. With 5% ppm manganese, acute chlorosis occurred at 15 ppm calcium (4622, 6622). High calcium levels gave better growth but the plants were still chlorotic (4222, 6222).

At low light intensity and low temperature (Plates VII and VIII) the symptoms were virtually absent, and spotting occurred only on three plants at pH 6, with low calcium and high manganese (6622). The difference in growth at pH 4 between the plants at high light intensity (Plate V) and low light intensity (Plate VII) is very marked.

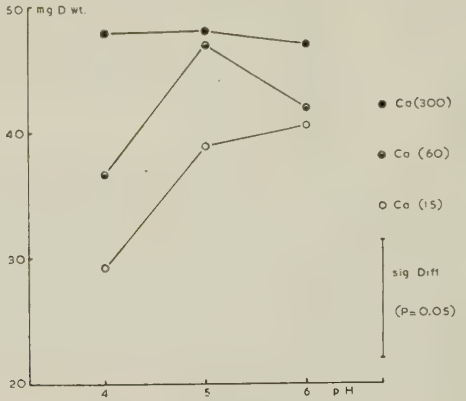


Fig. 6. The effect of increasing the calcium supply at the three pH levels on the yield of lucerne shoots at a high light intensity (Block I, Expt. 24).

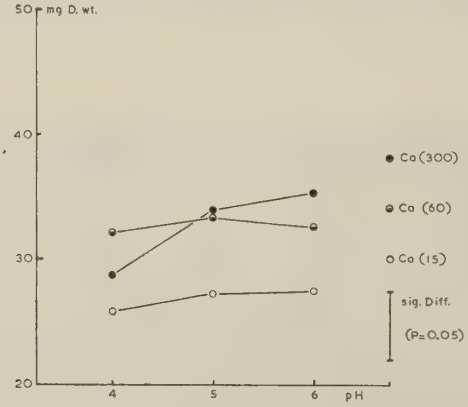


Fig. 7. The effect of increasing the calcium supply at the three pH levels on the yield of lucerne shoots at a low light intensity (Block II, Expt. 24)

of pH and calcium, and the mechanism of both this effect and the influence of temperature on root injury at pH 4, are not at present clear. An effect of climatic variables on hydrogen-ion toxicity is undoubted, and is probably the explanation for the seasonal effect of the toxicity of a low pH to plants which has been reported from time to time (*e.g.* for barley, Arnon [1]).

(b) *Toxicity of a high manganese concentration*

The toxicity of manganese was also found to be variable, and to be affected by pH, calcium level and by the light intensity.

Fig. 8 illustrates the effect that pH level had on manganese toxicity. Although the growth at the lower level of manganese was much better at pH 6 and pH 5 than at pH 4, the reduction in yield caused by the highest level of manganese (50 ppm) was much greater at the higher pH levels, and indeed the yield was actually

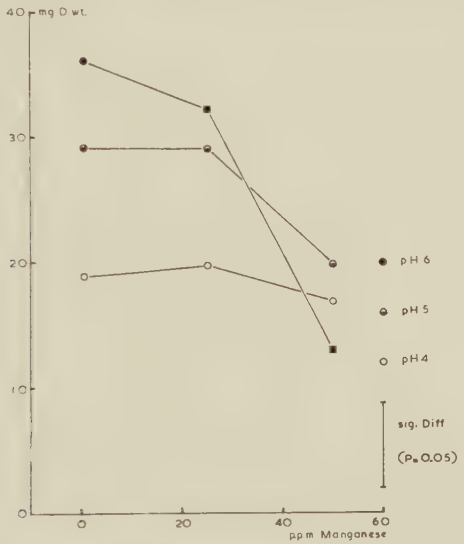


Fig. 8. The interaction between pH and manganese level on the yield of lucerne shoots (Block III, Expt. 24).

TABLE IV

The mean manganese content (as ppm D.Wt.) of the plants grown at the 50 ppm manganese level in Experiment 24 (see Table II)

Level	Blocks				Total mean
	I	II	III	IV	
pH 4	2847	1905	1965	2129	2211
pH 5	2577	1870	2817	2055	2330
pH 6	3141	2174	3448	2588	2838
Ca 0	3839	2386	3475	3039	3185
Ca 1	2701	1956	2865	2177	2425
Ca 2	2024	1607	1890	1557	1770
Total mean	2855	1983	2743	2257	

Sig. Diff. between means in body of table ($P = 0.05$) = 947.1
,, ,, ,, ,, ,, pH and Ca totals ,, = 473.6
,, ,, ,, ,, ,, block totals ,, = 546.8

lower at pH 6 than at pH 4. Although this is contrary to the usual findings of manganese uptake from acid *soils*, the results are in agreement with those of Olsen [12] who found a rise in manganese uptake over the pH range 3.5–6.5 with a subsequent fall at a higher pH, for several plant species, when grown in water culture. This fall in uptake above pH 6.5 may be the explanation of Hewitt's [4] result. He found manganese more toxic to swedes and mustard at pH 3.5–4.0 than pH 5.5–6.0, although when he discusses the interaction between manganese and aluminium he quotes a figure of 6.5–7.0 for the pH of the high manganese solution. In pure culture the cause of the increased uptake from pH 4 to pH 6 appears analogous to that of the increased calcium uptake under similar conditions, and is due to the increased ratio of the metal ions to hydrogen ions, at the higher pH level. In soil, however, the amounts of manganese in the divalent state, and hence available for plant uptake are so very different at the two pH levels that there is generally a much greater uptake of manganese from the more acid soil.

A high calcium level was also effective in reducing the toxicity of manganese to lucerne. The effect of the yield can be seen in Fig. 9, while the results of the chemical analyses which show the reduced manganese uptake with higher levels of calcium supply

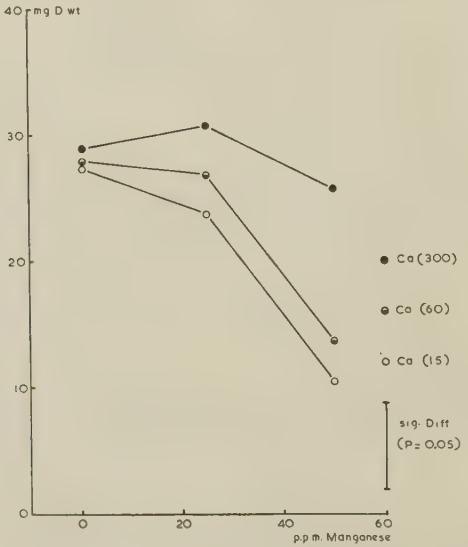


Fig. 9. The interaction between calcium and manganese level on the yield of lucerne shoots (Block III, Expt. 24).

are presented in Table III. This antagonism of calcium and manganese in agar culture is probably due to a direct competition between the two divalent ions for uptake, whereas the similar effect of a reduced manganese content of plants following the liming of an acid soil is more likely to be chiefly due to the effect of the lime on the soil pH and so on the amount of divalent manganese present in the soil.

The effect of light intensity upon manganese toxicity was very marked. Fig. 10 shows that the reduction in yield due to 50 ppm

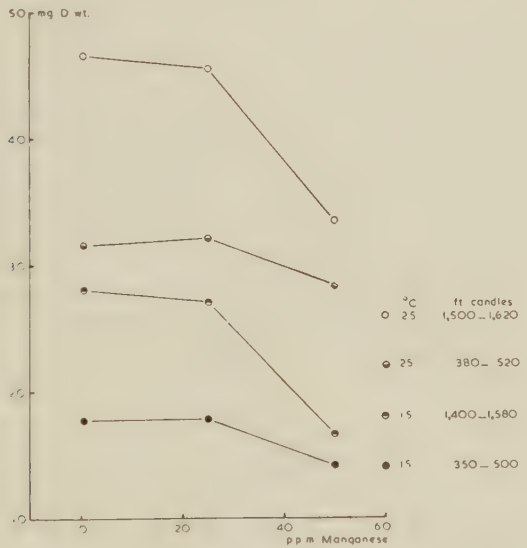


Fig. 10. The effect of the climatic treatments in Expt. 24 on the toxicity of manganese to lucerne.

manganese was much greater where the light intensity was high (Blocks I and III). Furthermore, at this high light intensity the symptoms of manganese toxicity that are usually obtained in these experiments (orange-brown spotting predominantly near the leaflet tips), were replaced where the toxicity was greatest, by a chlorosis. No chlorosis was observed at the low light intensity. (See Plates I–VIII). The chemical analysis of this plant material (Table IV) showed that the concentration of manganese within the plants at the high light intensity was greater than at the lower intensity, but that the differences were scarcely sufficient to explain the

considerable differences in symptoms and yields which were obtained Some further explanation of the effects of light intensity at a given manganese concentration is necessary to account fully for the facts observed. Gerretsen [3] has suggested that a high manganese level affected the iron-manganese ratio which caused an increased destruction of the chloroplasts under high light intensities. Although it is not known whether the chlorosis found in these experiments was indeed a symptom of iron deficiency, this evidence suggests that light intensity is an important factor in manganese toxicity and that it may well be that the importance of the iron: manganese ratio which has been stressed by Somers *et al.*[13] may depend upon the intensity of illumination. This may explain why some workers (*e.g.* Morris and Pierre [10 11], Hewitt [4] have been unable to confirm the importance of this ratio, while others working in lower latitudes (Hopkins *et al.* [6], Millikan [9]) have found it to be operative. In an outdoor experiment Löhnis [7] found chlorosis only appeared during spells of hot weather. Presumably, in this case the higher temperature would be accompanied by a higher light intensity, and it should be to this factor that differences could be attributed, as no effect of temperature was evident in the experiments described here.

SUMMARY

The effects were studied of hydrogen-ion concentration, calcium and manganese supply on the yield of lucerne in both agar and water culture. One agar experiment allowed the effects of two light intensities and two temperatures, on these factors, to be investigated.

The toxicity of pH 4 was found to vary, and calcium and nitrogen levels, culture method and climatic conditions were considered contributory factors. The high manganese supply was also found to vary in its toxicity, the most adverse effects being at pH 6, a low calcium supply and a high light intensity. Where the toxicity was most severe the usual orange-brown spotting symptoms were replaced by a chlorosis. Some chemical analyses of the manganese contents of the plants grown under these conditions are reported.

ACKNOWLEDGEMENT

Acknowledgement is made to the University of Nottingham for a Tomlin Scholarship (C.D.S.) during the tenure of which this work was undertaken.

Received September 4, 1957.

BIBLIOGRAPHY

1 Arnon, D. I., Ammonium and nitrate nitrogen nutrition of barley at different seasons in relation to hydrogen-ion concentration, manganese, copper and oxygen supply. Soil Sci. **44**, 91–121 (1937).

2 Arnon, D. I. and Johnson, C. M., Influence of hydrogen-ion concentration on the growth of higher plants under controlled conditions. Plant Physiol. **17**, 525–539 (1942).

3 Gerretsen, F. C., Manganese in relation to photosynthesis. III. Uptake of oxygen by illuminated crude chloroplast suspensions. Plant and Soil **2**, 323–343 (1950).

4 Hewitt, E. J., The resolution of the factors in soil acidity. The relative effects of aluminium and manganese toxicities on farm and market garden crops. Ann. Rept. Long Ashton Research Sta. **1947**, 58–65 (1948).

5 Hewitt, E. J., A biological approach to the problems of soil acidity. Intern. Soc. Soil Sci. Trans. Dublin 1952 **1**, 107–118 (1953).

6 Hopkins, E. F., Pagan, V. and Ramirez-Silva, F. J., Iron and manganese in relation to plant growth and its importance in Puerto Rico. J. Agr. Univ. Puerto Rico **28**, 43–101 (1944).

7 Löhnis, M. P., Manganese toxicity in field and market garden crops. Plant and Soil **3**, 193–222 (1951).

8 McCool, M. M., Effect of light intensity on the manganese content of plants. Contrib. Boyce Thompson Inst. **7**, 427–437 (1935).

9 Millikan, C. R., Effects on flax of a toxic concentration of B, Fe, Mo, Al, Cu, Zn, Mn, Co or Ni in the nutrient solution. Proc. Roy. Soc. Victoria **61**, 25–42 (1949).

10 Morris, H. D. and Pierre, W. H. The effects of Ca, P and Fe in the tolerance of lespedeza to manganese toxicity in culture solutions. Soil Sci. Soc. Am. Proc. **12**, 382–386 (1947).

11 Morris, H. D. and Pierre, W. H., Minimum concentrations of manganese necessary for injury to various legumes in culture solutions. Agron. J. **41**, 107–112 (1949).

12 Olsen, C., Uber die Manganaufnahme der Pflanzen. Biochem. Z. **269**, 329–348 (1934).

13 Somers, I. I., Gilbert and Shive. The iron-manganese ratio in relation to the respiratory CO_2 and deficiency-toxicity symptoms in soybeans. Plant Physiol. **17**, 317–320 (1942).

Plant and Soil IX, no 4 July 1958

EFFECT OF GIBREL, A POTASSIUM SALT OF GIBBERELLIC ACID, ON MICROBIAL ACTIVITIES IN SOIL *

by K. C. LU and W. B. BOLLEN

Department of Bacteriology, Oregon Agricultural Experiment Station, Corvallis

Gibberellic acid, a metabolic product from *Gibberella fujikuroi*, has been shown to greatly accelerate plant growth [2 4]. No information has been found concerning any direct effects on microbial activities in soils. A preliminary study from this laboratory showed that gibberellic acid (alcohol soluble) has no significant effect on terminal respiration of the soil microbial population in conventional Warburg runs [3]. It was observed, however, than an ethanolic gibberellic acid solution did effect some increase in numbers of *Azotobacter chroococcum*.

By the use of Gibrel ($C_{19}H_{21}O_6K$), a water-soluble potassium salt of gibberellic acid, Merck & Co. Inc., the complicating effects of ethanol were avoided in the following experiments on soil respiration, and on nitrogen fixation by *Azotobacter chroococcum*.

Triplicate fifty-gram portions (dry basis) of a sandy loam soil were placed in half-pint milk bottles of a respiration apparatus [1]. This soil had a pH of 7.2, and analyzed 0.06% N and 0.80% C. Treatments were made with Gibrel, glucose and reacted coffee grounds** (XlC) as indicated in Table I. The moisture content of each soil sample was adjusted to 50% of the water-holding capacity: of the required amount of water, a portion sufficient to slightly moisten the soil was mixed with the sample before transferring it to the bottle, after which the remainder of the water with the desired amount of Gibrel was added. The bottles were connected to the respiration apparatus and

* Technical Paper No. 1070. Oregon Agricultural Experiment Station, Corvallis.

** Spent coffee grounds from instant coffee manufacture; processed (Patent pending) to retard decomposability, and to increase analysis to 4.3-3-1 (N-P_2O_5-K_2O), giving a C : N ratio of 13/1.

slight pressure was used to pass CO_2-free air over the soil. The respiratory CO_2 was absorbed in $N/1$ NaOH and determined at intervals of 18 hours, 2, 4, 7, 14 and 30 days by double titration.

In the nitrogen-fixation experiment; quadruplicate 500-mg samples of air-dried soil taken from a previous Warburg experiment [3] were passed aseptically through a 40-mesh screen, and sprinkled evenly over the surface of petri plates containing 50 ml nitrogen-free agar medium [5]. The plates were then incubated at 30°C for 5 days. At the end of the incubation period, the amount of nitrogen fixed was determined by the Kjeldahl method. All results were analyzed statistically.

The rate and extent of CO_2 evolution from soil is a measure of decomposition for native as well as for added organic matter [1]. In the latter instance it is assumed that all CO_2 above the amount released by the untreated control soil originates from the organic addition. This is essentially true where the organic matter additions are not excessive, although any increased microbial activity can result in more decomposition of the native organic matter, and an addition of an essential trace element or growth substance to a soil which is deficient in such an element or substance, can stimulate the decomposition of the native organic matter considerably.

Terminal results for all treatments are shown in Table I. The Gibrel additions to the soil without other organic materials resulted in increased CO_2 production only after 4 to 7 days (Figure 1). Previous to this time there appears to have been a period of adaptation. Gibrel at 1000 ppm produced the greatest quantitative increase, but 5 ppm appeared most effective on the basis of the amount of Gibrel added, especially after 14 days. Data in Table I may be interpreted in two ways: 1) None of the Gibrel was decomposed to CO_2, in which case all the CO_2 must have resulted from decomposition of the native organic matter; 2) Some of the Gibrel was decomposed to CO_2 and to make up the total the balance must have originated from the soil organic matter. Although the prevailing mechanism cannot be resolved without resorting to labeled carbon, certain limited interpretations may be made.

In the first place it appears that Gibrel does stimulate decomposition of the native organic matter. Assuming complete decomposition of Gibrel at 5 and 50 ppm, there is still a significant, though small, excess of CO_2 that must have arisen from the soil. With the higher rates of Gibrel the total CO_2 evolved could account for complete decomposition of only part of the addition, or for partial

TABLE I

Effect of Gibrel on soil respiration and organic decomposition as shown by CO_2 production after 30 days incubation

Treatment	Carbon			Carbon as CO_2			Apparent Decomposition		
	In soil organic matter	In Gibrel	In added organic matter	Evolved in 30 days	Increase over control	Excess CO_2 over assumed complete Gibrel decomposition	Based on soil organic matter	Based on Gibrel	Based on added organic matter
	mg	mg	mg	mg	mg	mg	%	%	%
Soil only	400	—	—	8	—	—	2.0	—	—
Soil + Gibrel @ 1000 ppm	400	29.6	—	18	10	—	2.5*	34.3	—
Soil + Gibrel @ 100 ppm	400	3.0	—	10	2	—	0.5*	71.3	—
Soil + Gibrel @ 50 ppm	400	1.5	—	12	4	2.5	1.0*	100.0	—
Soil + Gibrel @ 5 ppm	400	0.2	—	11	3	2.8	0.8*	100.0	—
LSD ($P = .05$)				1.5					
Soil only	400	—	—	8	—	—	1.9	—	—
Soil + glucose @ 2000 ppm C	400	—	100	113	105	—	—	—	100.0
Soil + glucose + Gibrel @ 100 ppm	400	3.0	100	91	83	80	—	—	83.1
Soil + glucose + Gibrel @ 50 ppm	400	1.5	100	96	88	86.5	—	—	88.5
Soil + glucose + Gibrel @ 5 ppm	400	0.2	100	91	83	82.8	—	—	82.8
LSD ($P = .05$)				5.6					
Soil only	400	—	—	8	—	—	1.9	—	—
Soil + reacted coffee grounds @ 2000 ppm C	400	—	100	37	29	—	—	—	29.2
Soil + reacted coffee grounds + Gibrel @ 100 ppm	400	3.0	100	31	23	2.0	—	—	23.5
Soil + reacted coffee grounds + Gibrel @ 50 ppm	400	1.5	100	37	29	27.5	—	—	28.8
Soil + reacted coffee grounds + Gibrel @ 5 ppm	400	3.2	100	41	33	32.8	—	—	33.2
LSD ($P = .05$)				2.2					

* Increase over control

decomposition of a greater amount. Since the 5- and 50-ppm treatments increased decomposition of native organic matter, it can be assumed that the 100- and 1000-ppm rates had a more pronounced similar effect, or at least some of the CO_2 in excess of the control could well be derived from the native organic matter. The possible complete decomposition of the Gibrel at 5 and 50 ppm is in agreement with Brian's result in England [2], showing that gibberel-

lic acid in unsterile soil rapidly lost its activity, probably being decomposed by soil microorganisms. Inactivity may result from partial decomposition, however, with little or no CO_2 production.

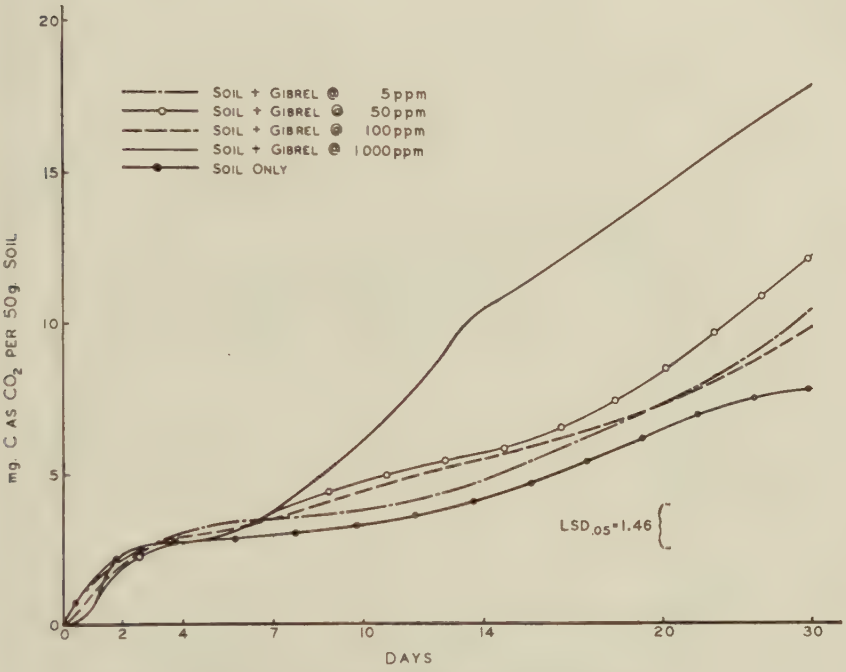


FIG. 1 CUMULATIVE CO_2 EVOLUTION FROM SOIL + GIBREL

With added glucose equivalent to 2000 ppm carbon (Figure 2), CO_2 production was highest and at 30 days indicated complete decomposition of this substrate. The incorporation of Gibrel decreased the rate of CO_2 evolution from glucose, the differences becoming established at 2 to 4 days, after which they were maintained. No significant difference was shown at 30 days between 5 and 100 ppm, and 50 and 100 ppm. However, there was significant difference between 5 and 50 ppm Gibrel. The results offer no explanation for the decreased CO_2 production in the presence of Gibrel. Possibly more glucose was assimilated by the soil microflora, or it was more efficiently utilized. Evidence for greater assimilation was obtained in the nitrogen-fixation study.

With addition of Gibrel to the reacted coffee grounds, a resistant organic material having a C : N ratio of 13/1, and in these respects similar to native soil organic matter, the CO_2 production was

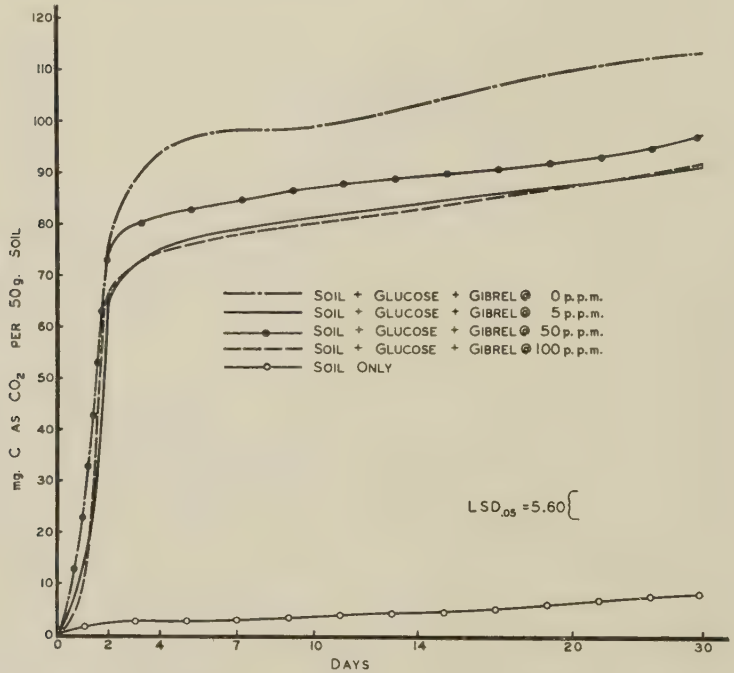

mg. C AS CO_2 PER 50g. SOIL
DAYS
SOIL + GLUCOSE + GIBREL @ 0 p.p.m.
SOIL + GLUCOSE + GIBREL @ 5 p.p.m.
SOIL + GLUCOSE + GIBREL @ 50 p.p.m.
SOIL + GLUCOSE + GIBREL @ 100 p.p.m.
SOIL ONLY
$LSD_{.05}$ =5.60

FIG. 2 CUMULATIVE CO_2 EVOLUTION FROM SOIL + GLUCOSE @ 2 000 p.p.m. C + GIBREL

decreased with Gibrel at 100 ppm and increased at 5 ppm (Figure 3). The 50 ppm addition had no significant effect. These effects with coffee grounds are qualitatively similar and quantitatively intermediate to those shown with glucose on the one hand and soil organic matter on the other. Based on these results, as well as on results with soil, and with soil plus glucose, it seems that Gibrel of 5 and 50 ppm represent a critical dosage range for major effects on CO_2 production from either native or added organic matter.

The effect of Gibrel on the nitrogen fixation by *Azotobacter chroococcum* is shown in Table II. The results reveal that in all cases the Gibrel additions gave significant increases in nitrogen fixation, the maximum effect being at 50 ppm. There was little difference between nitrogen fixation with Gibrel at 1, 3 and 5 ppm; 1000 ppm

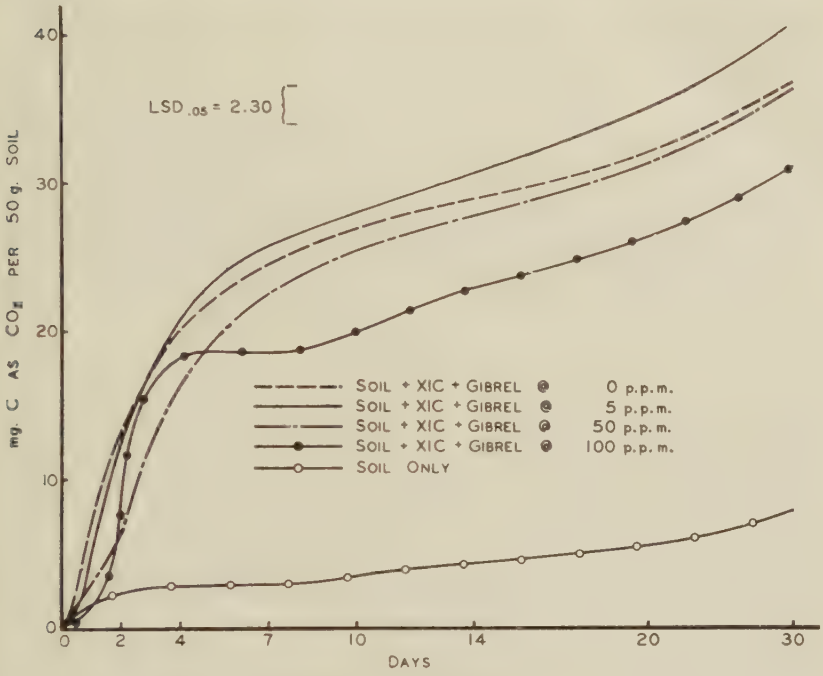


FIG. 3 CUMULATIVE CO_2 EVOLUTION FROM SOIL + XIC @ 2000 p.p.m. C + GIBREL

TABLE II

Effect of Gibrel on nitrogen fixation by *Azotobacter chroococcum* on nitrogen-free agar plates inoculated with 500 mg soil, incubated 5 days at 30°C

Treatment	Total nitrogen mg
Soil only	0.05
Soil + Gibrel @ 1 ppm	10.15
Soil + Gibrel @ 3 ppm	9.20
Soil + Gibrel @ 5 ppm	9.67
Soil + Gibrel @ 50 ppm	11.56
Soil + Gibrel @ 1000 ppm	11.06
$LSD_{.05}$ =	0.524

gave no significant difference from 50 ppm. While effect of Gibrel could have been on the nitrogen-fixing process, it seems more likely that cell numbers were increased, since the lesser amounts of CO_2 evolved from glucose in the presence of Gibrel could indicate the synthesis of more cells. *Azotobacter* development on all the plates was so heavy that no reliable estimate of numbers could be made.

In view of results given by lower concentrations it appears unlikely that the Gibrel served as an energy source.

Gibrel contains approximately 10 per cent potassium, and *Azotobacter* is particularly reponsive to deficiencies in available forms of this element. However, in view of the abundant growth of *Azotobacter* in the control soil as well as with the various treatments, it appears unlikely that the small amounts of potassium added in the Gibrel had any influence on microbial development.

SUMMARY

From respiration studies with a neutral sandy loam soil, it appears that Gibrel can speed up decomposition of native and added organic matter. The results with Gibrel treatments alone indicate the gibberellic molecule may be rapidly decomposed by soil microbes. Evidence is presented to show that this moiety stimulates nitrogen fixation by *Azotobacter* in the soil. The critical range for these effects of Gibrel appeared to be 5 to 50 ppm. These results were obtained with a single soil type. It remains to be investigated if similar indications would occur with other soil types where some factors of microbial environment may be quite different.

Received July 31, 1957.

REFERENCES

1 Bollen, W. B., Soil respiration studies on the decomposition of native organic matter. Iowa State Coll. J. Sci. **15**, 353–374 (1941).

2 Brian, P. W.. Elson, G. W., Hemming, H. G. and Radley, Margaret, The plant-growth-promoting properties of gibberellic acid, a metabolic product of the fungus *Gibberella fujikuroi*. J. Sci. Food Agr. **5**, 602–612 (1954).

3 Lu, K. C., Gilmour, C. M., Zagallo, A. C. and Bollen, W. B., Preliminary investigation of gibberellic acid effects on soil microorganisms. Nature (London) **181**, 189–190 (1958).

4 Marth, P. C., Audia, W. V. and Mitchell, J. W., Effects of gibberellic acid on growth and development of plants of various genera and species. Botan. Gaz. **118**, 106–111 (1956).

5 Martin, W. P., Distribution and activity of *Azotobacter* in the range and cultivated soils of Arizona. Arizona Agr. Expt. Sta. Tech. Bull. **83** (1940).

Plant and Soil IX, no 4 July 1958

THE PROPERTIES OF A BIOLOGICALLY FORMED MANGANESE OXIDE, ITS AVAILABILITY TO OATS AND ITS SOLUTION BY ROOT WASHINGS

by S. M. BROMFIELD

Division of Plant Industry, C.S.I.R.O., Canberra, A.C.T. Australia

I. INTRODUCTION

Whilst it is well known that manganous ions oxidize rapidly in soils above pH 5.5, and especially in those between pH 6 and 7, the properties of the oxides so formed are virtually unknown since it is impossible to separate them from the soil. This oxidation was thought by some to render manganese unavailable to plants but it is now agreed that the oxides so formed, as well as other forms of manganese oxide are available to plants (Leeper [14], Jones and Leeper [12], Fiskel and Mourkides [7], and Heintze [9]). The relative availabilities of the biologically formed oxides and manganous ion however are not known and it has not been ascertained to what extent oxidation decreases manganese availability.

The availability of active manganese oxides in soils is reported to be mainly due to their reduction by soil organic matter, which is favoured by hydrogen ions (Leeper [14]). Reduction by micro-organisms can also occur (Mann and Quastel [17]). The direct solution of manganese oxide by plants has not been studied, although speculation on this has been made by Jones and Leeper [13] and Beckwith [2 3].

An opportunity of examining these problems arose when it was found possible to obtain relatively large amounts of a biologically formed manganese oxide (Bromfield [4]). The present paper describes studies of the properties of the oxide, its availability to oats, and its solution by root washings.

II. METHODS AND MATERIALS

Preparation of biologically formed manganese oxide

Manganese medium, pH 6.0, was inoculated with a suspension of *Corynebacterium* sp. (Bromfield [4]). After 20 h at 27°C, when the first sign of oxidation appeared, the supernatant of the cultures was decanted into sterile beakers and incubated at 27°C, for a further 2 days when the oxide settled out. The decanting procedure was necessary to prevent the oxide sample becoming grossly contaminated with the calcium phosphate present in the manganese medium. The oxide deposit was harvested by centrifugation, washed once in distilled water and stored as an aqueous suspension in stoppered bottles.

Weight of bacterial cells in oxide deposit.

It was observed (Bromfield [4]) that the deposit which formed in liquid cultures was an intimate mixture of cells and manganese oxide. The amount of cells present was determined by dissolving a known weight of deposit in a solution of sulphur dioxide, collecting the cells by centrifugation, washing them with water and then vacuum drying them in aluminium foil dishes. The dried cells were then weighed. The loss of weight on heating of these cells to 120°C and 250°C was also determined.

Properties of the manganese oxide.

Total manganese in the deposit was determined colorimetrically as permanganate (Piper [19]).

"Available oxygen" or "MnO_2" in the deposit was determined by reduction with oxalic acid and back-titrating residual oxalic acid with 0.05*N* potassium permanganate. In this determination correction was made for bacterial cell contamination. The correction was obtained by titrating equivalent amounts of oxide-free cells with permanganate.

The value *of* n in the formula MnO_n was calculated from the results obtained above.

Solubility of the oxide in water, 1% acetic acid and *N* sulphuric acid was determined on 17 mg of deposit in 100 ml of solvent at 20°C for 1 h. The acetic acid solubility was taken as a measure of water insoluble manganous impurities *e.g.* carbonate or phosphate.

Reduction by neutral aqueous quinol (0.05% w/v) after 1 h contact was determined on 17.2 mg deposit in 50 ml quinol solution to obtain a chemical estimation of the manganese supplying potential of the oxide for plant growth (Jones and Leeper [12]).

Availability of the oxide to oats.

The availability of manganese oxide to Algerian oats was determined in a manganese-deficient soil, in acid-washed quartz sand, in nutrient agar and in nutrient solution. The oats were grown in 500 ml pots and in 6″ × 1″ test tubes. The details of the manganese treatments are given in the Results section.

The soil, a manganese-deficient black friable clay (pH 6.9) from Penola, South Australia, received a basal addition of dry superphosphate, potassium nitrate, magnesium sulphate, copper sulphate and zinc sulphate at rates equivalent to 8, 2, 0.5, 0.1, and 0.1 cwt/ac respectively. Four hundred grams of this soil was added to weighed pots and moistened with 120 ml of glass-distilled water. Appropriate quantities of bacterial manganese oxide and manganous sulphate were thoroughly mixed with the moist soil and then two pregerminated Algerian oats from manganese-deficient plants were sown in each pot. All treatments were in triplicate. The pots were placed in a glasshouse and their water content adjusted daily to field capacity. The plants were harvested at maturity and the dry weight of the tops, the number of grains and the manganese content of the whole top of the plants were determined (Piper [19]).

For studies in quartz sand, 600 g of acid-washed sand was initially moistened with 120 ml of manganese-free nutrient solution prepared from recrystallized salts. The nutrient solution used was essentially that of Arnon [1] except, that ferric chloride replaced ferrous tartrate because it had been shown that ferrous tartrate can dissolve manganese oxides (Bromfield [5]). An additional 350 ml of nutrient solution was given to each pot during the growing period and the water content was adjusted daily with glass-distilled water. The pH of the sand cultures was measured with a glass electrode at the beginning and end of the experiment. In other respects the experimental procedure was the same as for Penola soil.

Availability studies under sterile conditions were conducted in 6″ × 1″ test tubes using Thornton's technique [21]. It was changed slightly by using the modified Arnon's nutrient solution mentioned above. The oat seeds were sterilized by washing in alcohol for 60 sec under vacuum and then in 7% w/v calcium hypochlorite for 15 min followed by 6 rinses with sterile distilled water. Ten tubes were used for each treatment and the treatments were replicated 5 times. The plants were grown in a shaded glasshouse at 20°C to 25°C and after 40 days the tubes were tested for sterility. The sterile plants were harvested and the dry weight and manganese content of the tops determined. The pH of the nutrient medium was also determined.

III. RESULTS

a) *Properties of the bacterial manganese oxide*

Examination of the bacterial manganese oxide by X-ray and electron diffraction failed to distinguish any crystalline manganese oxides. The oxide is apparently amorphous. It was observed that the oxide shrank to about one sixth of its moist volume during vacuum drying at air temperature and at the same time changed from dark brown to black. This shrinkage suggested that the manganese oxide might be hydrated, so loss of weight on heating was determined. In determining this it was necessary to allow for the

loss of weight of the bacterial cells present in the bacterial manganese oxide. Accordingly the weight of the cells and their loss on heating were also determined. The loss of weight of the manganese oxide present in the sample could then be calculated by difference. It was found that the bacterial manganese oxide sample, vacuum dried at 35°C, contained 22.6 per cent bacterial cells and 77.4 per cent manganese oxide. It lost 46.5 per cent of its weight when heated to 250°C. This loss was due to loss from the bacterial cells (13.4 per cent) and to loss from the manganese oxide (33.1 per cent). The manganese oxide fraction in the sample thus lost 43 per cent of its weight when heated from 35°C to 250°C. It is suggested that this loss is mainly due to loss of water of hydration.

The manganese composition of the deposit was next examined. The absolute amounts of manganese and "MnO_2" in 17.2 mg of the bacterial manganese-oxide deposit were 4.7 mg and 6.55 mg respectively, representing 27.3 per cent manganese and 38.0 per cent "MnO_2". The calculated value of n in the formula MnO_n was 1.88. This figure of 1.88 indicated that the manganese was fairly completely oxidized, since a value of 2 is the maximum found for insoluble manganese oxides. If, as suggested above, the heat-labile fraction of this oxide is mainly water of hydration, then 43 per cent H_2O in the formula $MnO_{1.88}.nH_2O$ would be accounted for by 3.6 moles of hydration. Values as low as 1.76 for n were obtained for other preparations of bacterial manganese oxide.

The above determinations showed that the bulk of the content of the vacuum-dried bacterial manganese-oxide sample could be accounted for in the following way — cells 22.6 per cent, "MnO_2" 38.0 per cent, excess Mn as "MnO" (calculated) 4.3 per cent, heat labile 33.1 per cent, unaccounted 2.0 per cent. In this balance sheet it was assumed that the manganese in excess of that in the "MnO_2" was present as "MnO". To test whether there was gross contamination of the bacterial manganese-oxide sample with water-insoluble manganous salts, the solubility of the oxide was determined in 1 per cent acetic acid and in water. Its solubility was measured also in N sulphuric acid. There was no measurable water-soluble manganese, 1.6 per cent of the toal manganese was acetic acid soluble and 21.3 per cent of the total was sulphuric acid soluble. The low acetic-acid-soluble fraction suggests that the oxide was relatively free from manganous-salt impurities.

The result of the chemical estimation of availability of the oxide deposit using neutral quinol showed that 33 percent of the manganese in the oxide was reduced after 1 h. The percentage of the total manganese reduced could however be increased by increasing the volume of quinol solution used. The ease of reduction suggested that the oxide should be available to plants (Jones and Leeper [12]). To confirm this, plant-availability studies were next undertaken.

b) *Plant-availability studies*

Jones and Leeper [12] have shown that some manganese oxides can cure manganese deficiency in Penola soil, so this soil was used for availability studies. The treatments given to the soil were a no manganese control, 2.5 ppm manganese as manganous sulphate and 25 ppm manganese as the dried oxide. It was found during the course of the experiment that the 2.5 ppm manganese as manganous sulphate was insufficient to cure grey speck on this soil and it was necessary to keep adding manganese to keep the plants healthy. The soil received during the growing period a total of 17.5 ppm manganese. The plants were grown for 26 weeks from June 3 to December 1, 1954. Grey-speck symptoms developed in the no-manganese control after 3 weeks and these plants remained diseased. Grey speck also appeared on plants which received the oxide. These plants grew much better than the controls but they were poorer than those on the manganous sulphate treated soil. The results of this experiment are given in Table I.

TABLE I

Dry weight of tops, number of grains and manganese content of 26 weeks old Algerian oats grown in Penola soil receiving various manganese additions. Sown 3. VI. 1954

Addition	ppm Mn	μg Mn/pot	Dry weight of tops g/pot	Number of grains per pot	Mn in tops μg/pot (log)
$MnSO_4$	17.5	7000	4.48	45.7	71 (1.83)
Bact. Mn oxide	25	10000	2.72	24.7	24 (1.37)
Nil			0.82	1.3	13 (1.10)
L.S.D. 5%			0.66	7.5	(0.27)
1%			1.00	11.3	(0.41)

These results indicated that the oxide was partly available but was less available than manganous sulphate since the plant yields and the amount of manganese absorbed by the plants were less.

The amount of added manganese extracted from the soil by the plants was very small being about 0.8 per cent in the case of manganous sulphate and 0.1 per cent in the case of the oxide. The difference in availability of the two manganese sources might be due solely to the fact that the manganous sulphate was applied in small amounts throughout the growing period, whereas all of the manganese oxide was applied as the dried oxide at the time of planting.

The large amounts of manganous sulphate required to overcome manganese deficiency in this soil compared with the small amounts required to grow healthy plants in nutrient solution (Samuel and Piper [20]) indicated that the Penola soil rapidly rendered most of the soluble manganese unavailable A possible reason for this loss of availability was that the soluble manganese rapidly became oxidized to an unavailable oxide, since relatively large amounts of bacterial manganese oxide did not cure manganese deficiency. Alternatively, something else in the soil limited the availability of both manganous sulphate and manganese oxide. To test these possibilities, availability studies were made in sand cultures. An experiment was set up to compare the availability of the manganese oxide suspension with that of manganous sulphate. The treatments were replicated five times and consisted of a no-manganese control, 0.4 ppm Mn as manganous sulphate and 0.4 ppm and 4.0 ppm Mn as the oxide suspension. The manganese concentrations are expressed as ppm of the sand. The oxide suspensions containing known amounts of manganese were added to the sand drop by drop and then thoroughly mixed.

Another comparison of availability was made between the moist and vacuum-dried oxide deposit at the 4-ppm level. This was included to determine whether drying affected the availability of the oxide since, as mentioned above, the oxide underwent considerable shrinkage during drying. The dry oxide was added to sand in two ways: (a) by adding a weighed amount of powdered, vacuum-dried oxide deposit to the sand and mixing thoroughly and (b) by applying the oxide suspension drop by drop to the sand, mixing thoroughly and then vacuum drying the mixture. In the first case the oxide consisted of discrete particles throughout the sand whereas in the second case the oxide formed a thin coating over many of the sand grains and thus presented a larger oxide surface to plant roots. The pots were then treated as described under Methods.

The plants were grown for 21 weeks from August 15, 1955 to January 12, 1956. Grey-speck symptoms appeared in the no manganese control plants after 10 weeks and at harvest the plants grown on the 0.4-ppm level of both manganese sources appeared identical. The determinations made at harvest are presented in Table II.

TABLE II

Dry weight of tops, number of grains and manganese content of 21 weeks old Algerian oats grown in sand receiving various manganese additions. Sown 15. VIII. 1955

Addition	ppmMn	μg Mn/pot	No. of replicates	Dry wt. tops g/pot	No. grain per pot	Mn in tops μg/pot (log)
$MnSO_4$	0.4	240	5	7.84	96.6	221 (2.34)
Bact. Mn oxide	0.4	240	5	8.00	93.2	262 (2.41)
Bact. Mn oxide	4.0	2400	5	7.41	76.0	2298 (3.36)
Bact. Mn oxide a) dried before mixing	4.0	2400	3	7.30	76.0	621 (2.79)
Bact. Mn oxide b) dried after mixing	4.0	2400	3	7.78	79.6	2381 (3.37)
Nil			5	3.33	15.8	35 (1.52)
L.S.D. 5% between 5 and 5 reps				0.62	19.6	0.09
5 and 3 reps				0.72	22.7	0.11
3 and 3 reps				0.80	25.4	0.12

The oxide was apparently as available as manganous sulphate since, at the 0.4-ppm level, the manganese contents of the plants, the plant weights and grain numbers were similar. Comparing the manganese contents of plants grown on the three 4-ppm treatments, it appeared that the manganese in the oxide was less available when it was dried before mixing with the sand. The reason for this was probably due to the smaller surfaces area of oxide exposed to the roots. The manganese content of the tops of the plant accounted for virtually all the manganese added to the pots in all treatments except the 4-ppm manganese added as the previously dried oxide sample. To confirm this, the amounts of manganese present in 150-g samples of sand from the various treatments were determined after harvest. It was found that manganese was detectable only in the sand which received the dried oxide, and that the amount present accounted for that not in the plant. This showed that the oxide suspension was completely available to oats except when it was dried before mixing with the sand.

It was thought that the complete availability of the moist manganese oxide may have been related to the formation of acid conditions during the growth of the plant, however, pH determinations revealed that the pH rose in all treatments from an initial value of 6.0 to a final value which varied from 6.7. to 7.0 within treatments. This suggested that the plant roots themselves or their associated rhizosphere micro-organisms were mobilizing the oxide.

To determine which of these were important in mobilizing manganese oxide, its availability was studied in test tubes under sterile and unsterile conditions. The treatments consisted of a no-manganese control, and 15 μg of manganese per test tube as manganous sulphate, as bacterial manganese oxide and as γ-MnO_2.

TABLE III

Yield and manganese content of oats grown in test tubes under sterile and unsterile conditions for 40 days on various sources of manganese

Manganese source	Yield per plant (mg)		Mn content of 10 plants (μg)			
	Sterile	Unsterile	Sterile	(logs)	Unsterile	(logs)
Nil	29.9	29.5	3.5		0.0	
$MnSO_4$	28.1	31.0	85.7	(1.96)	55.5	(1.74)
Biol. Mn Oxide	29.1	30.0	51.7	(1.71)	36.3	(1.56)
γ-MnO_2	28.5	29.3	31.0	(1.49)	20.7	(1.32)
L.S.D. 5%	N.S.	N.S.		0.06		0.06
1%				0.08		0.08

The results of the determinations at harvest are given in Table III. It is apparent that the ability of the plant to obtain manganese from manganese oxide is a property of the plant itself since the sterile plants obtained appreciably more manganese than did the unsterile plants.

There was no yield difference within the manganese treatments nor between them and the control. Excluding the nil treatment the mean yield for the sterile plants is significantly less than for the unsterile plants, the interaction of manganese treatments with sterility being non significant. In contrast to this the manganese contents of the plants showed highly significant differences between all treatments within sterile and unsterile plants. The interaction of sterility with manganese treatments is not significant, but the increases in manganese content with sterilizing are highly significant. It appears that the plants obtained manganese from

manganous sulphate at a faster rate than from the oxides and that, under the conditions of the experiment, unsterile conditions adversely affected this rate.

The pH determinations at harvest showed that the unsterile plants were growing in a medium which was 1pH unit more alkaline (pH 8.5) than the sterile plants (pH 7.5). It was probable that this higher pH adversely affected the ability of the unsterile plant to obtain manganese. It was also possible that micro-organisms decomposed substances released from the plant roots which could dissolve manganese oxides.

c) *Studies on the solution of manganese oxide by plants*

An experiment was made to determine whether oat roots formed substances which could dissolve manganese oxide or whether intact roots were also required. Sterile oat plants were grown in test tubes containing manganese-free nutrient solution and after 28 days the solutions were collected. Manganese oxide was added to heated and unheated samples of this solution and, after 6 hours, qualitative tests for manganese were made on filtrates of the suspension. It was found that manganese oxide was dissolved by both solutions, thus indicating that neither intact roots nor enzymes were required for the solution process. It was found subsequently that the root washings of unsterile, 20-week-old, oat plants which had been grown in sand, could dissolve manganese oxide. This result suggested that the substances responsible are fairly resistant to microbial decomposition or are being continuously released in moderately large amounts.

Since the availability of manganese is markedly affected by pH, a study was made of its effect on the dissolving power of the root washings. These were obtained by soaking the roots of 30 6-week-old oat plants in 800 ml of distilled water for 2 h and then centrifuging the liquor at 6,000 rpm for 10 minutes. A 20 ml aliquot of the supernatant was then added to 1 ml of 0.5 per cent aq γ-MnO_2 suspension and 5 ml of various buffer solutions, which ranged from pH4 to pH9. The acid buffers were prepared from 0.2*N* sodium acetate and acetic acid, and the neutral and alkaline ones from 0.05*M* sodium borate and 0.2*M* boric acid. The mixtures were left for 24h at 35°C and then manganese determined in 20 ml of the filtered supernatant. The results of these determinations

are presented in Figure 1. Buffer controls without washings did not dissolve any detectable amounts of manganese. The dissolving power of the washings was inversely related to the pH of the mixture, that is it increased with decrease in pH. The amount of manganese dissolved above pH 6.5 was very small.

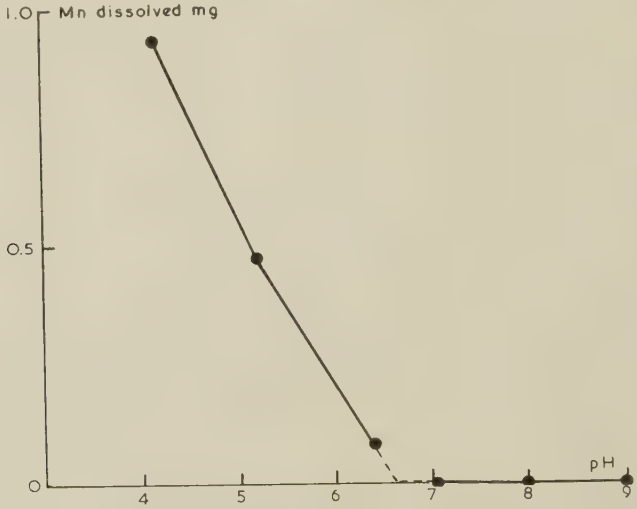


Fig. 1. Showing the effect of pH on the solution of γ-MnO_2 by washings of oat roots.

It was thought possible that the low availability of manganese oxide in Penola soil was due to substances in it which inactivated the root washings. To test this, 20 ml of root washings were added to 5 g Penola soil and, after 6 h at room temperature, they were collected by centrifugation and the ability of 15 ml of this supernatant to dissolve manganese oxide at pH 5 was compared with 15 ml of untreated washings. It was not possible in this way to detect any loss of dissolving ability of the root washings.

IV. DISCUSSION

The biological oxidation of manganese does not seem to decrease its availability to oats grown in sand culture, unless the oxide is subsequently dried and thus added as relatively large particles (Table II). In soil it is possible that applications of manganous

ions are oxidized only at certain points of microbial activity which results in their being precipitated as relatively large particles of limited availability to plant roots.

Jones and Leeper [13] suggest that the poor residual effect of various manganese compounds, including manganous sulphate, on some soils is most likely to be due to the formation of unreactive, orderly structured crystals of manganese oxide. It seems from the present work, however, that another process may be involved since it is unlikely that the biologically formed oxide aged so rapidly and completely in the Penola soil and not at all in the sand (Tables I and II). That the oxide is amorphous and intimately mixed with bacterial cells also indicates that crystallization would not proceed rapidly if in fact at all

The finding that oat roots release substances which can dissolve manganese oxide and that the activity of these is dependent on pH enables the problem of manganese availability to be considered in a new light. It is well known that the availability of manganese in soils is decreased by liming and increased by acidifying (Mulder and Gerretsen [18], p. 229), and this has been attributed to the effect of pH on the reducing power of soil organic matter (Leeper [14]). However it was difficult to interpret these effects in soils low in organic matter. It seems now that they may have been due to the fact that the solution of manganese oxide by root substances is dependent on pH (Fig. 1).

The results of the study of manganese availability in test tubes (Table III) indicate that the manganese from the manganous sulphate was taken up more rapidly than that from the oxide. While it is admitted that these plants were grown under extremely artificial conditions, it is probable that the result obtained also applies to plants grown under more natural conditions. Jones and Leeper [12] found that the rate at which various manganese oxides were reduced by quinol gave a good index of their availability. Thus the rate at which plants dissolve manganese oxide is important in determining its availability. At present it is not known whether the root substances dissolve the oxide by reduction or complex formation or by both these processes.

It is probable that the roots of various plants produced substances which vary both in kind and quantity, and this could account for the differing abilities of plants to obtain manganese

from soils (Samuel and Piper [20], Löhnis [16]). The ability of plant roots to lower the pH of the rhizosphere, which would be effected by the buffering capacity of the soil, would also increase the solution of oxides.

It is suggested above that the low availability of added manganese compounds in Penola soil is not due to the ageing of an oxide. Present results show that it is not due to compounds in soil which compete with manganese oxide for the substances from roots. It seems likely that the root substances dissolve the manganese oxide in Penola soil, but that soil colloids successfully compete with the roots for the small amounts that are dissolved at a pH near neutrality, and that they tightly hold the manganese. That this is possible is supported by the results of Heintze and Mann [8 9], Hemstock and Low [11] and Beckwith [3] which indicate that some soils contain organic colloids which can form insoluble complexes with manganese. Little appears to be known of the factors which govern the activity, properties and occurrence of manganese complexing organic colloids in soils, but it seems that the manganese in them is eventually oxidized since, much of the manganous manganese added to soils high in organic matter ends up as the oxide (Beckwith [3]).

The amount of manganese available to a plant in near neutral and alkaline soils appears to be the net result of the rates of two opposing processes, those of solution and fixation. To the many factors which affect the rate of solution of manganese oxide (Leeper [14 15], Dion, Heintze and Mann [6], Mulder and Gerretsen [18], Jones and Leeper [12]), that of the nature and amount of manganese dissolving root substances must be added.

SUMMARY

Manganese oxide, produced by *Corynebacterium* sp. in liquid medium was found to be amorphous, probably hydrated and was readily reduced by neutral quinol. Preparations of the oxide had values of n in the formula MnO_n which ranged from 1.76 to 1.88. The oxide was completely available to oats grown in sand culture but only slightly available in a manganese deficient soil. Plants grown under sterile conditions on agar slopes were able to obtain manganese from manganese oxide, indicating that the roots and not associated micro-organisms, were responsible for the solution process. Root washings of oat plants contained substances which dissolved manganese oxides and the activity of these substances increased with increasing acidity. The possible importance of these substances in making soil manganese available to plants is discussed.

ACKNOWLEDGEMENT

The author wishes to acknowledge Mr. G. A. McIntyre, Section of Mathematical Statistics, C.S.I.R.O. for the analyses of the data in Tables I, II and III, and Dr. L. H. P. Jones for the gift of γ-MnO_2 and sample of Penola soil

Received July 23, 1957

REFERENCES

1 Arnon, D. I., Microelements on culture-solution experiments with higher plants. Am. J. Botany **25**, 322–324 (1938).
2 Beckwith, R. S., Manganese in soils and its uptake by plants Australian Conf. Soil Sci. **1**, 2.17.1–2.17.6 (1953).
3 Beckwith, R. S., Metal complexes in soils. Australian J. Agr. Research **6**, 684–698 (1955).
4 Bromfield, S. M., Oxidation of manganese by soil micro-organisms. Australian J. Biol. Sci. **9**, 238–252 (1956).
5 Bromfield, S. M., The solution of manganese oxide by iron sources used in nutrient solutions. Plant and Soil **8**, 389–394 (1957).
6 Dion, H. G., Mann, P. J. G., and Heintze, S. G., The easily reducible manganese of soils. J. Agr. Sci. **37**, 17–23 (1947).
7 Fiskel, J. G. A. and Mourkides, G. A., A comparison of manganese sources using tomato plants grown in marl peat and sand soils. Plant and Soil **6**, 313–331 (1955).
8 Heintze, S. G. and Mann, P. J. G., Studies on soils manganese Part 1 Pyrophosphate as extractant of soil manganese J. Agr. Sci. **39**, 80–95 (1949).
9 Heintze, S. G. and Mann, P. J. G., A study of the various fractions of the manganese in neutral and alkaline organic soils. J. Soil Sci. **2**, 234–242 (1951).
10 Heintze, S. G., The effects of various soil treatments on the occurrence of marsh spot in peas and on manganese uptake and yields of oats and timothy. Plant and Soil **7**, 218–236 (1956).
11 Hemstock, G. A. and Low, P. F., Mechanisms responsible for retention of manganese on the colloidal fraction of soil. Soil Sci. **76**, 331–343 (1953).
12 Jones, L. H. P. and Leeper, G. W., The availability of various manganese oxides to plants. Plant and Soil **3**, 141–153 (1951).
13 Jones, L. H. P., and Leeper, G. W., Available manganese oxides on neutral and alkaline soils. Plant and Soil **3**, 154–159 (1951).
14 Leeper, G. W., Forms and reactions of manganese in the soil. Soil Sci. **63**, 79–94 (1947).
15 Leeper, G. W., Factors affecting availability of inorganic nutrients in soils with special reference to micro-nutrient metals. Ann. Rev. Plant Physiol. **3**, 1–16 (1952).
16 Löhnis, Marie P., Manganese toxicity in field and market garden crops. Plant and Soil **3**, 193–222 (1951).
17 Mann, P. J. D. and Quastel, J. H., Manganese metabolism in soils. Nature **185**, 156 (1946).
18 Mulder, E. G. and Gerretsen, F. C., Soil manganese in relation to plant growth. Advances Agron. **4**, 222–272 (1952).
19 Piper, C. S., Soil and Plant Analysis. University of Adelaide, Adelaide (1942).
20 Samuel, G. and Piper, C. S., Manganese as an essential element for plant growth. Ann. Applied Biol. **16**, 493–524 (1929).
21 Thornton, H. G., The early development of the root nodule of lucerne (*Medicago sativa* L.). Ann. Botany (London) **44**, 385–92 (1930).

Plant and Soil IX, no. 4 July 1958

A STUDY OF THE MECHANISM OF SOIL-PHOSPHATE UPTAKE IN RELATION TO PLANT SPECIES

by P. H. NYE and W. N. M. FOSTER

University College of Ghana

INTRODUCTION

In the classical investigations that have been made into the ability of different plant species to utilise sparingly soluble soil phosphates or applied phosphates, the criterion by which a plant's performance has been judged has been its total uptake of phosphorus or its increased yield when an insoluble fertilizer was applied. Marais [11] has reviewed early work on this subject and Thomas [28], Smith [25] and Lyness [10] have reported that different species or even varieties of plants vary in the efficiency with which they are able to extract from the soil the less readily available forms of soil phosphorus. Russell [18] in a discussion of the subject mentions lupins as one of the strongest extractors of phosphate among farm crops and quotes Schander [23] who suggests that they solubilize less readily available phosphate by excreting an organic acid from the roots. It is also known that plants vary greatly in their uptake of phosphorus from added sparingly soluble phosphates such as basic calcium phosphate or iron and aluminium phosphates (see reviews by Fried and Dean [5] and Kurtz [8]). In general it has been found that the Leguminosae and Cruciferae can use sparingly soluble phosphates to a greater extent than the Gramineae or Solanaceae. Among the beneficial effects attributed to green manure crops such as lupins or sweet clover is their partial effectiveness in using the less soluble soil phosphates and returning them to the soil through their leaves in a more soluble form.

The question of the phosphorus-extracting efficiency of different

species is of special interest in the study of humid tropical soils. Fertility in most of these soils is restored by the natural resting fallow. The 'available' phosphate status of many of these soils as measured by conventional chemical extractants is often extraordinarily low and thus the possibility of finding a plant species which is particularly effective in utilising relatively insoluble phosphates and releasing them through the litter in a more available form is of considerable agricultural interest.

It is clear that differences between the phosphate content of plants grown under identical conditions could be due to differences in the area of their root absorbing surfaces or to differences in the efficiency of the plant in extracting the phosphate from the soil. It is impossible to measure the root absorbing surfaces of plants growing in soil, partly because it is very difficult to recover all the fine roots, and partly because it is not known what portion of the root is actually absorbing. Differences in the efficiency of plants in extracting the different forms of phosphate from the soil have been attributed to the production of carbon dioxide (Parker [17]), and the effects of the rhizosphere population (Dalton [2]).

It was considered that further understanding could be obtained about the differences in the extracting efficiency of plants by growing a range of plant species under identical conditions in pots containing soil into which carrier-free P^{32} had been thoroughly mixed. It seemed that the measurement of the ratio of P^{32}/P^{31} in the plant might indicate whether the different species were all using the same forms of soil phosphorus. For example, a plant that was able to feed on a very insoluble form of soil phosphate might be expected to have a higher proportion of P^{31} : P^{32} (*i.e.* a lower specific activity) than a plant unable to use such forms. The results for two of the soils used have been briefly reported (Nye and Foster [16]).

Since there was no information on the effect of radiation on plant growth and phosphate uptake under conditions in which the P^{32} is intimately mixed with the whole soil, studies to determine the importance of such effects were also made.

EXPERIMENTAL METHODS

Soils

Soils used in the experiments were: —

(a) Pokoase brown loamy coarse sand developed from granite. Neutral, of low phosphate status.

(b) Achimota red sandy loam developed from quartzite. Ferruginous, acid, of very low phosphate status.

(c) Bunsu yellowish brown clay developed from greenstone, a basic metamorphosed lava. Slightly acid, of low phosphate status. The phosphate content of these soils, fractionated by the method of Williams [31], and the available phosphate determined by the Truog method [29] is shown in Table I.

TABLE I

Fractionation of phosphate (ppm P) in Pokoase, Achimota and Bunsu soil

Soil	Pokoase	Achimota	Bunsu
pH (Soil: water 1:2)	7.21	5.01	5.66
Organic C, per cent	0.97	0.75	1.23
2.5% acetic acid 1% oxine sol. P	24	8	7
0.1 *N* NaOH sol. inorg. P	13	12	30
Organic P	51	43	74
Total P	169	131	287
„Available" P	18.5	3.6	3.3

Plant species

Members of the Leguminoseae (groundnuts, pigeon pea, soyabean and centrosema), Cruciferae (Cauliflower), Gramineae (maize, millet and sorghum) and Solanaceae (tomato) were used. The amount of phosphate taken up from these soils of low phosphate status was in the early experiments not sufficiently in excess of the seed phosphate of the large seeded plants. Since it is likely that a small but unknown amount of the phosphate originally in the seed will exchange isotopically with phosphate in the soil, the interpretation of the results in these cases suffers from a measure of uncertainty. In the later experiments attention was concentrated on small-seeded plants in order to reduce this source of error.

The preparation of labelled soil

The soils exployed were air dried, passed through a 4-mm sieve and each thoroughly mixed to ensure homogeneity. For the Pokoase soil a Kenwood domestic mixer was used to incorporate the tracer with the soil.

Preliminary trials indicated that with 1350 g of soil and 50 ml of tracer solution, added slowly from a pipette while mixing, a five minute mixing period gave an adequate distribution of P^{32} as determined by solid counting on small portions of soil. In experiments where large quantities of soil were employed (Achimota and Bunsu soil) the technique was modified in that a high-activity solution was mixed with a subsample of the soil in the Kenwood mixer, and this subsample was then incorporated in a correspondingly larger volume of soil in a soil-mixing drum. Rotation of the drum for fifteen minutes gave satisfactory mixing of the unlabelled and labelled soil. Before mixing, stock P^{32} solutions were made up to 1 ppm P strength with KH_2PO_4. This reduced adsorption of P^{32} onto glassware but when incorporated into the soil the level of the carrier phosphate (0.037 micrograms P/g soil and not 0.002 micrograms P/g soil as previously reported by Nye and Foster[16]) was so low that the *P* status of the soils was virtually unchanged. The P^{31} added could therefore be ignored.

Activities of P^{32} employed

In the first experiment with Pokoase soil the possibility of radiation damage affecting the results was considered. Four activities of P^{32} were therefore employed (0, 5, 20 and 100 μC P^{32} per litre of soil). The soil receiving no P^{32} was given an identical mixing treatment with distilled water.

Since the levels of radiation caused no significant differences in the specific activities of the plants, in all further experiments approximately 10–15 μC P^{32} per kilogram of soil was selected as the most convenient dose rate.

Sampling procedure

In all experiments roots and shoots were sampled separately. Shoots were cut off at the soil surface and the pots soaked in 2% lead-acetate solution for 12 hours. This procedure reduced the loss of phosphate when the roots were subsequently washed free from soil in a sieve submerged in water (Russell and Adams[20]). Visual examination of the soil after washing indicated that root losses by breakage were low. Small root and shoot samples were dried *in toto* at 100° C for 24 hours. Large samples were dried, ground in a mill and a subsample redried for analysis.

Analysis and assay procedure

Analyses were carried out on approximately 300 mg of dried plant material. Extracts for analysis were prepared by the wet-ashing technique using 1 ml of 60% perchloric acid and a variable quantity of pure nitric acid (1–5 ml) depending on the nature and amount of the plant material. When the ashing process was complete the extract was filtered and diluted to a volume of 10 cc.

Estimation of P^{32}

In all experiments the liquid counting technique described by Martin and Russell[12] using type M. 6. Geiger Muller counter tubes was employed to measure radioactivity.

Estimation of P^{31}

After counting, total phosphate (*i.e.* P^{31}) was determined colorimetrically as molybdivanadophosphoric acid (Kitson and Mellon [7]) using a Spekker photo-electric absorptiometer.

EXPERIMENTAL RESULTS

It is convenient to express the results of the determinations of specific activity in the plants in terms of the "Larsen" value (L) (Russell *et al.*,[22] and Larsen [9]) which is defined by the expression

$$L = \frac{P^{31} \text{ plant} - P^{31} \text{ seed}}{P^{32} \text{ plant}} \times (P^{32} \text{ added to soil}) \qquad (1)$$

and is expressed as ppm P in the soil. In expressing the results in terms of L values we do not at this stage wish to imply any specific mechanism for isotopic dilution either in plant or soil.

Radiation effects

In previous work that has been carried out on the effects of radiation on plant growth, the P^{32} has been applied to a water culture (Martin and Russell [13]) or has been applied in solution or as labelled fertilizer to the surface or to a definite zone in the soil (see Mattingly [14] for references). These experiments, while establishing that there are significant radiation effects on the growth and phosphate uptake of plants, do not show consistent trends. Conditions are very different when P^{32} is uniformly mixed throughout the whole mass of soil.

Experiment 1 was designed to investigate the effects of radiation from various levels of tracer phosphate in the soil on the growth, phosphate uptake, and specific activity of plants, and also to obtain preliminary information on the variation in specific activity between different species. The results are recorded in Table II.

Effect on dry weight

After 40 days growth sporadic damage effects were apparent in the dry weights. The dry weights of the shoots of groundnuts treated with 5 μC and 20 μC P^{32}, and of millet treated with 5 μC P^{32} were significantly lower than the controls. In contrast the dry weight of the maize roots showed a significant increase

TABLE II

The effect of P^{32} radiation (per cent of control) on the dry weight and P uptake of plants harvested after 40 days. Five replicates of two plants per pot

Plant species	Control (mg per pot)	μC P^{32} in 1.3 kg soil			Sig. Diff. $P = 0.05$
		5	20	100	
Dry weight					
Shoots					
Groundnuts	5241	85.6*	86.2*	94.3	9.9
Maize	4671	97.4	97.9	100.8	8.2
Soyabean	4038	92.4	90.8	97.7	10.2
Millet	4868	91.1*	94.9	100.6	8.3
Pigeon pea	3104	96.3	100.6	105.0	13.3
Roots					
Groundnuts	2372	93.2	103.4	94.6	14.3
Maize	3443	111.3	112.2*	118.7*	12.0
Soyabean	1655	98.7	96.4	103.0	9.9
Millet	2037	88.7	99.4	103.0	13.0
Pigeon pea	1778	89.9	100.0	95.8	11.9
P uptake					
Shoots					
Groundnuts	6.703	88.3	93.8	93.8	11.8
Maize	4.784	100.8	105.7	99.7	10.1
Soyabean	6.451	83.1*	82.6*	81.1*	12.3
Millet	5.983	63.8*	73.1*	76.6*	15.0
Pigeon pea	6.502	72.1*	78.5*	82.6*	14.4
Roots					
Groundnuts	3.689	78.6	83.5	82.3	21.4
Maize	3.268	95.5	94.0	97.7	14.2
Soyabean	2.461	101.1	97.9	101.2	12.6
Millet	1.897	87.9	97.8	91.1	16.5
Pigeon pea	3.199	81.4	86.1	83.3	22.3

* Difference from control significant at $P = .05$

in the 20 μC and 100 μC P^{32} treatments. Although only isolated figures are significant there does appear to be a trend in the results in that with the exception of maize roots there is a tendency for the lowest dry weights of roots and shoots to occur in the 5 μC P^{32} treatment. At the two higher levels of P^{32} the plant weights approximate more closely to the control values.

Effect on P uptake

The data for the phosphate contents of the plants demonstrate that with the exception of maize which showed no significant

difference between treatments the phosphate contents were significantly lower than the controls in all three treatments. This decrease in phosphate content was caused largely by a reduction in the shoot phosphate content. The results indicate that the plants employed were susceptible to radiation injury at levels of P^{32} varying from 5–100 μC P^{32} per litre of soil, but that the effects of injury were not constant between plant species. The most consistent indication of injury appeared to be the decreased phosphate content in root and shoot.

Effect on specific activity

The L values calculated from Equation (1) at the different levels of activity are given in Table III. It is apparent that the variation in activity has no effect on the calculated value for any of the crops and there is therefore no likelihood of radiation injury invalidating the conclusions to be drawn from this value.

TABLE III

L value (ppm) after 40 days growth in 1.3 kg of Pokoase soil treated with three concentrations of P^{32} (5 replicates of 2 plants per pot)

μC P^{32} per 1.3 kg soil	Ground-nut	Maize	Soyabean	Millet	Pigeon pea	Treat-ment mean.
5	19.0	24.0	20.8	23.2	21.1	21.6
20	21.6	24.0	20.8	22.4	21.5	22.1
100	21.6	23.2	20.7	23.6	22.4	22.3
Crop Mean	20.7	23.7	20.8	23.1	21.7	

Sig. Diff. $P = 0.05 = 1.5$ for individual crop means
$P = 0.05 = 1.1$ for treatment means

The effect of the different crops on the L value

In Table IV the effects of various crops on the L value in the three soils as determined in Experiment 1 and three subsequent experiments are presented. For reasons explained in the next section the plants are arranged in decreasing order of seed P content.

The effect of seed phosphate

In Experiment 1 it appeared that the L value was lower when the P in the seed was an appreciable fraction of the P uptake, due

TABLE IV

L values obtained from a variety of crops

Expt. No. and Soil	Expt. period	Plant	P. content (mg)	P. seed (mg)	L value (ppm)
1 Pokoase	40	Groundnut	4.59	1.64	20.7
5 replicates of 2 plants/pot (1.3 kg soil) averaged over activity levels of 5, 20, 100 μC per 1.3 kg soil	days	Soyabean	3.88	0.64	20.8
		Maize	4.01	0.57	23.7
		Pigeon Pea	3.87	0.40	21.7
		Millet	3.00	0.07	23.1
		Sig. Diff.	$P = .05$		1.5
2 Pokoase	40	Centrosema	1.61	0.07	22.8
10 replicates of 1 plant/pot (1.3 kg soil) at 20 μC per 1.3 kg soil	days	Sorghum	1.95	0.04	26.7
		Cauliflower	1.08	0.03	26.9
		Tomato	1.81	0.01	25.5
		Sig. Diff.	$P = .05$		3.0
3 Achimota	60	Groundnut	5.74	2.11	7.9 ± 0.2
5 replicates of 1 plant/pot (17 kg soil) at 10 μC per/kg soil	days	Soyabean	1.92	0.46	9.2 ± 0.5
		Pigeon Pea	3.92	0.34	9.9 ± 0.6
		Millet	5.37	0.04	10.4 ± 0.3
		Sorghum	0.58	0.04	13.1 ± 2.7
4 (A) Bunsu	58	Pigeon Pea	3.24	0.40	39.1
8 replicates of 1 plant/pot (2 kg soil) at 15 μC per kg soil	days	Millet	5.59	0.07	45.0
		Sorghum	3.69	0.04	45.2
		Cauliflower	2.27	0.03	42.5
		Tomato	1.01	0.01	40.0
		Sig. Diff.	$P = 0.05$		4.84
4 (B) Bunsu	97	Pigeon Pea	10.39	0.40	38.4
8 replicates of 1 plant/pot (2 kg soil) at 15 μC per kg soil	days	Millet	13.52	0.07	40.5
		Sorghum	14.34	0.04	40.2
		Cauliflower	9.37	0.03	39.7
		Tomato	5.26	0.01	38.9
		Sig. Diff.	P 0.05		4.64

to exchange between seed phosphate and P^{32} added to the soil. In later experiments steps were taken to ensure that the P seed was reduced to small proportions by using small seeded plants. Even so the phosphate status of the Achimota soil was so poor that it was difficult to ensure a sufficiently large uptake of soil phosphorus, and in pots containing one kilogram of soil the growth of soyabeans, maize, pigeon pea and to a lesser extent millet after 30 days was so poor that in some of the plants there was a net loss of phosphate during the experiment. Since the plants contained an appreciable amount of P^{32}, phosphate must have

exchanged between the seedling and the soil. The method finally adopted for obtaining a greater uptake of P from very deficient soils (Expt. 3) was to use 17 kg of soil and only one plant per pot.

It may be noted that under these conditions the addition to the soil of a labelled phosphate fertilizer instead of carrier free P^{32} does not greatly help because it does not greatly increase the amount of soil P, in distinction to fertilizer P, that is taken up.

Thus, when an amount F of labelled fertilizer phosphate is added to the soil the equation for the calculation of L, assuming no exchange between soil and seed P, is

$$L + F = (P^{31} \text{ plant} - P^{31} \text{ seed}) \times \frac{P^{32} \text{ in soil}}{P^{32} \text{ in plant}} \qquad (2)$$

If there is complete exchange between soil and seed P, the equation is

$$L + F = (P^{31} \text{ plant}) \times \frac{P^{32} \text{ in soil}}{P^{32} \text{ in plant}} \qquad (3)$$

The value of *L* lies between the values calculated from these two equations, and the uncertainty is given by

$$\Delta L = (P^{31} \text{ seed}) \times \frac{P^{32} \text{ in soil}}{P^{32} \text{ in plant}}$$

which from equation (3) reduces to

$$\Delta L = \frac{L + F}{P^{31} \text{ plant}} \times P^{31} \text{ seed}$$

The uncertainty in the calculation of L will therefore only be reduced by adding fertilizer to the soil if the addition causes a greater proportionate rise in (P^{31} plant) than in (L + F). This is possible for small fertilizer additions in very phosphate deficient soils, as shown by Dean's yield-of-phosphate curves[3]. It corresponds to the lower part of the typical sigmoid curve relating phosphate uptake to phosphate supply. The small reduction in ΔL is however offset by a greater absolute analytical error in the determination of P^{31} plant as this quantity is increased.

These conclusions may be illustrated by the results of an experiment in

Crop	Total P in plant (mg)	P seed (mg)	L value (ppm)	
			Assuming no exchange with seed	Assuming complete exchange with seed
Carrier-free P^{32}				
Maize	1.141	0.385	8.78 ± 0.40	13.25 ± 0.61
Pigeon pea	0.572	0.335	7.40 ± 1.36	17.9 ± 3.30
50 ppm P^{31}				
Maize	10.252	0.385	7.31 ± 1.00	8.80 ± 1.20
Pigeon pea	4.774	0.335	4.43 ± 0.40	7.12 ± 0.64

which maize and pigeon pea were grown in 1.3 kg of the phosphate-deficient Achimota soil with addition of carrier-free P^{32}, and with the same level of P^{32} together with potassium dihydrogen phosphate equivalent to 30 ppm of soil.

Although the uncertainty in L has been somewhat reduced by adding 30 ppm of P^{31}, any small constant error in the determinations is greatly magnified when L is determined as a difference between two large quantities.

Effects of plant species

If the uncertainty introduced by exchange of seed P is allowed for it will be noted that in all the soils the L values calculated from the specific activity of each of the species are approximately the same. The high value for sorghum in Experiment 3 is due to a large experimental error introduced by its exceedingly poor growth.

DISCUSSION

It must be concluded from these experiments that in neutral and acid soils the proportions of soil phosphorus to added phosphorus taken up by a range of plants is much the same for a given soil in spite of very great differences in the growth of the plants and their total uptake of phosphorus, and in spite of the fact that they may be phosphate starved.

There is some evidence for this conclusion from the experiments of other workers. Fried [6] found no difference in the proportion of fertilizer to soil phosphorus taken up by seven different crops grown in three soils into which labelled monocalcium phosphate had been mixed. The calculation of the proportions of soil and fertilizer phosphate taken up involve the same assumptions as those made in calculating Fried and Dean's [4] A values, and the result implies constant A values for these soils irrespective of species. The A value that is determined in these experiments is virtually the same as the L value in this paper, though the interpretation placed on the value differs as discussed below. The fact that fertilizer phosphate was used in Fried's experiments does not matter. The L value can be determined from Equation (2) when labelled soluble phosphate is mixed with the soil and it has been shown that the value is the same as that obtained when carrier free P^{32} is added (Russell *et al.*[22]). These workers also found a similar L value for both rye and barley on three different soils, but on a fourth soil of high phosphate adsorption capacity the value for rye greatly exceeded that of barley. Since in this experiment the uptake of P per pot by the barley was only 0.25 mg, a figure which must be comparable to the phosphorus content of a single seed, and more than one seed may have been used per pot, it is possible that exchange of phosphorus between the seed and the soil would be responsible for this greatly reduced L value.

The interpretation of the apparent constancy of the proportion of soil phosphorus to added phosphorus taken up may now be considered. There are, broadly, two approaches to the problem of the phosphate status of a soil in its relation to plant growth, and the experimental results presented in this paper may be discussed from either viewpoint.

I. For many years it has been considered that phosphorus which may be utilized by plants exists in the soil in several chemical or adsorption forms, which together constitute the 'available' phosphate. These available forms are considered to be of varying solubility (availability), plants which are weak phosphate feeders utilizing the more soluble forms, and plants which are strong phosphate feeders utilizing some of the less soluble forms as well. It is known that when P^{32} is added to the soil isotopic exchange occurs with part of the total soil phosphate at a varying rate, although, in sum, there is a rapid initial exchange of P^{32} followed by a very much slower exchange (McAuliffe *et al.*[15], Wiklander[30], Russell *et al.*[21]. The added P^{32} might exchange either with only part of the available soil phosphate, or with the whole of it, or with some of the unavailable phosphate as well. If exchange between added P^{32} and the available forms of phosphate was only partial the constant ratio of soil P to added P^{32} taken up by plants implies that they are all feeding on the available but unexchanged forms at the same rates. This is considered most unlikely and is evidence against the assumption of only a partial exchange with available forms. If the added P^{32} exchanges with all the available forms then every available form will have a constant specific activity and the specific activity of plants feeding on these forms will likewise be constant. The possibility that the added P^{32} may exchange with some unavailable forms as well is equally compatible with a constant specific activity in the plants. In this case the L value as defined by Equation (1) will be greater than the available P, and a constant L value is no evidence that the different forms of phosphate are equally available to all plants. Another expression of these conclusions is that plants do not use non-exchangeable forms of soil phosphorus (*i.e.* non-exchangeable during the growing period) and they do not do this even under conditions of acute P shortage.

If the initial rapid exchange is considered to take place with „surface" phosphate then the statement commonly made that certain plants are able to make phosphorus more available because of their ability to utilize the less soluble soil phosphates, needs to be qualified for neutral and acid soils by the observation that it is only further surface phosphate that they are utilizing, together possibly, with the limited amount of „internal" phosphate that accounts for the slower exchange reaction after the initial rapid one.

Fried and Dean [4] in discussing their concept of 'A value' regard the two sources of phosphate – the soil and the added labelled standard – as essentially independent. They consider that isotopic dilution may take place within the plant rather than, as suggested by Larsen [9], in the soil. If this is the case, and the 'A' value is assumed to be a measure of the available soil P related to a standard, then a constant 'A' value irrespective of plant species implies that the ratio of the amounts of the added labelled soluble phosphate and the amounts of soil phosphate taken up does not vary with the species. As pointed out already, this seems inherently improbable and calls into question the idea that isotopic dilution occurs in the plant rather than the soil when the added soluble phosphate is well mixed with the soil.

II. Schofield [24] has suggested that a fraction of the soil P exists in a "labile pool" which will maintain a definite equilibrium potential in the soil solution. This labile pool is equivalent to the rapidly exchangeable soil phosphorus. Plants absorb their phosphorus from this "pool", and if the pool contains added P^{32} they will take it up in the ratio of $P^{32} : P^{31}$ in the pool, since the P^{32} will have exchanged rapidly with the soil P in the pool. The constancy of the specific activities of the plant species employed in the experiments described in this communication would accord well with the "labile phosphate pool" theory.

An exact equivalence between labile P determined from the specific activity of plants and laboratory determinations of rapidly exchangeable P cannot be expected since some of the applied P^{32} will, during the growing period of the plant, exchange with some of the less readily exchangeable soil phosphate, and it is possible that the activity of the P absorbed early in the life of the plant will be slightly higher than that absorbed later. No evidence of this

effect was found in experiment 4 where harvests were carried out on two occasions (58 and 97 days). It is possible that in this soil the exchange reactions were completed by the time of the first harvest (58 days). Provided, however, that all the experimental plants are grown for the same period, and their P uptake follows approximately the same pattern with time, they would all be expected to show the same specific activity.

The constant specific activity of the different species investigated indicates that the plants have no means, in practice, of increasing significantly the size of the labile pool of soil phosphate under the experimental conditions described. Such means are a theoretical possibility. The plants might, for instance, be thought to have very locally in the rhizosphere such a high concentration of iron complexing anions (Swenson *et al.*[26]) that not only the exchangeable soil phosphorus but also normally unexchangeable forms would be released into the labile pool for immediate uptake, *e.g.* Talibudeen [27] has shown that increasing concentrations of citrate ions increase the amounts of exchangable soil P. These experiments indicate that this does not happen; though complexing ions in the rhizosphere may well lead to increased phosphorus uptake by plants because they increase the potential of the phosphate in the labile pool. There was clear evidence, especially in Experiment 3 on the very deficient Achimota soil that a plant such as millet was able to take up far more phosphorus than sorghum. Since root growth was not restricted in the large volume of permeable sandy soil employed, it is apparent that some species are more efficient than others in the absorption of "low potential" soil phosphate. This may be due to an ability to take up phosphate rapidly at very low concentration in the soil solution, or to an ability to raise the potential by formation of complexes in the rhizosphere.

SUMMARY

The concept of plant 'available' and 'unavailable' soil phosphate has been examined by growing a range of plant species in soils well mixed with carrier free P^{32}.

It was found that although activities of 5–100 μC per kg soil caused changes in dry matter and P uptake, they had no influence on the specific activity per unit dose.

Only small and agriculturally insignificant differences have been found in

the proportion of soil P to added P^{32} taken up by the different species in the acid and neutral soils employed. It is considered that such differences as there are may be due to exchange of phosphate between seed and soil. Marked differences, however, occurred in the total phosphate absorbed by the crops.

These results support the view that a fraction of the soil phosphate exists in a labile pool which will exchange with added P^{32} and will maintain a definite equilibrium potential in the soil solution. Plants do not appear to have a means of increasing significantly the size of the labile pool under the experimental conditions described. The disparity, however, between the total phosphate uptake especially in very deficient soils does suggest that certain species are more efficient than others in the absorption of soil phosphate at low potential.

ACKNOWLEDGEMENTS

The authors wish to acknowledge the advice given by Dr. R. S. Russell, Department of Agriculture, Oxford University, during the course of the work, and the assistance of Dr. A. H. Ward, Department of Physics, University College of Ghana, in electronic assays.

Received July 19, 1957.

BIBLIOGRAPHY

1 Barbier, G., Introduction to the study of soil phosphates. Trans. Sixth Intern. Congr. Soil Sci. Paris **B**, 387 (1956).
2 Dalton, J. D., Russell, G. C., and Sieling, D. H., Effects of organic matter on phosphate availability. Soil Sci. **73**, 173 (1952).
3 Dean, L. A., Yield-of-phosphorus curves. Soil Sci. Soc. Am. Proc. **18**, 462 (1954).
4 Fried, M., and Dean, L. A., A concept concerning the measurement of available soil nutrients. Soil Sci. **73**, 263 (1952).
5 Fried, M., and Dean, L. A., Soil and fertilizer phosphorus. *In:* Agronomy Vol. IV p. 55. Academic Press Inc., New York (1953).
6 Fried, M., The feeding power of plants for phosphates. Soil Sci. Soc. Am. Proc. **17**, 357 (1953).
7 Kitson, R. E., and Mellon, M. G., The colorimetric determination of phosphorus by the molybdivanadophosphoric acid method. Ind. Eng. Chem. Anal. Ed. **16**, 379 (1944).
8 Kurtz, L. T., Soil and fertilizer phosphorus. *In:* Agronomy, Vol. IV, p. 78. Academic Press Inc., New York (1953).
9 Larsen, S., The use of P^{32} in studies on the uptake of phosphorus by plants. Plant and Soil **4**, 1 (1952).
10 Lyness, A. S., Varietal differences in the phosphorus capacity of plants. Plant Physiol. **11**, 665 (1936).
11 Marais, J. S., The comparative agricultural value of insoluble mineral phosphates of aluminium, iron, and calcium. Soil Sci. **13**, 355 (1922).
12 Martin, R. P., and Russell, R. S., Studies with radioactive tracers in Plant

Nutrition. II. The estimation of radioactive tracers. J. Exptl. Botany **1**, 141 (1950).

13 Martin, R. P., and Russell, R. S., Studies with radioactive tracers in plant nutrition. III. The effect of radiation from phosphorus–32 on the growth and phosphate utilization of barley seedlings. J. Exptl. Botany **5**, 91 (1954).

14 Mattingly, G. E. G., The use of the isotope P^{32} in recent work on soil and fertilizer phosphorus. Soils and Fertilizers Commonwealth Bur. Soil Sci. **20**, 59 (1957).

15 McAuliffe, C. D., Hall, N. S., Dean, L. A., and Hendricks, S. B., Exchange reactions between phosphates and soils: Hydroxylic surfaces of soil minerals. Soil Sci. Soc. Am. Proc. **12**, 119 (1947).

16 Nye, P. H., and Foster, W. N. M., Do plants vary in their ability to utilise less readily available soil phosphate? Trans. Sixth Intern. Congr. Soil Sci. Paris **D**, 257 (1956).

17 Parker, F. W., Carbon dioxide production of plant roots as a factor in the feeding power of plants. Soil Sci. **17**, 229 (1924).

18 Russell, E. W., Soil Conditions and Plant Growth, p. 461. Longmans Green & Co., London, New York & Toronto (1952).

19 Russell, R. S., and Martin, R. P., The problem of radiation damage in fertilizer studies with labelled phosphate. Trans. Fourth Intern. Congr. Soil Sci. Amsterdam **2**, 138 (1950).

20 Russell, R. S., and Adams, S. N., The removal of plant roots from soil for the estimation of their phosphate content. Plant and Soil **5**, 223 (1954).

21 Russell, R. S., Rickson, J. B., and Adams, S. N., Isotopic equilibria between phosphates in soil and their significance in the assessment of fertility by tracer methods. J. Soil Sci. **5**, 85 (1954).

22 Russell, R. S., Russell, E. W., Marais, P. G., and Foster, W. N. M., Factors affecting the availability to plants of soil phosphates. Proc. Intern. Conf. Peaceful Uses Atomic Energy Geneva **12**, 103 (1955).

23 Schander, H., The displacement of optimum soil reaction during development of *Lupinus luteus*. Bodenk. u. Pflanzen ernähr. **20**, 129 (1941).

24 Schofield, R. K., Can a precise meaning be given to "Available" soil phosphorus? Soils and Fertilizers Commonwealth Bur. Soil Sci. **18**, 373 (1955).

25 Smith, S. N., Response of inbred lines and crosses in maize to variations of nitrogen and phosphorus supplied as nutrients. J. Am. Soc. Agron. **26**, 785 (1934).

26 Swenson, R. M., Cole, C. W., and Sieling, D. H., Fixation of phosphate by iron and aluminium and replacement by organic and inorganic ions. Soil Sci. **67**, 3 (1949).

27 Talibudeen, O., Isotopically exchangeable phosphorus in soils. Part II. Factors influencing the estimation of 'labile' phosphorus. J. Soil Sci. **8**, 86 (1957).

28 Thomas, W., The feeding power of plants. Plant Physiol. **5**, 443 (1930).

29 Truog, E., The determination of the readily available phosphorus of soils. J. Am. Soc. Agron. **22**, 874 (1930).

30 Wiklander, L., Kinetics of phosphate exchange in soils. Ann. Roy. Agr. Coll. Sweden **17**, 407 (1950).

31 Williams, C. H., Studies on soil phosphorus. I. A method for the partial fractionation of soil phosphorus. J. Agr. Sci. **40**, 233 (1950).

Plant and Soil IX, no 4 July 1958

COMPETITION FOR SULPHUR IN A GRASS-CLOVER ASSOCIATION

by T. W. WALKER and A. F. R. ADAMS

Canterbury Agricultural College*,
University of New Zealand.

INTRODUCTION

Responses of pastures to sulphur have been obtained in Canterbury and other areas of the South Island [7], and evidence has been presented [8] indicating that the primary cause is low atmospheric return of sulphur compounds. It has already been suggested [5] that when most of the sulphur is present in the organic matter, as is the case in the A horizon of well drained soils, in humid-temperate climates, with fairly constant N : S ratios, grasses will utilise virtually all the mineral nitrogen and sulphur made available from the microbial decomposition of organic matter. Associated clovers will thus usually be dependent on atmospheric or fertilizer sources of sulphur if they are to fix nitrogen symbiotically.

In a pasture, the suppression of legumes which usually follows the stimulation of grasses by nitrogen fertilizers may be due to competition for light, moisture or nutrients. Blaser and Brady [2] have shown that applications of potassium fertilizers may lessen the suppression of clovers when nitrogen is applied to a grass-clover association grown on a soil deficient in potassium; Trumble and Shapter [1] obtained similar results with phosphorus. The Canterbury foothills area, where negligible amounts of sulphur appear to be obtained from the atmosphere, giving rise to a condition of acute sulphur deficiency, is ideal for the study of competition for sulphur by grasses and clovers in the presence and absence of fertilizer nitrogen.

* Postal address: Lincoln College, Christchurch, N.Z.

EXPERIMENT AND METHODS

The soil type was Kowai sandy loam, a recent loessial soil, situated on a high river terrace near the Rakaia Gorge, on the same farm but a mile or more from previous experimental sites [7 8]. The soil (0" – 4") analyzed as follows:

Soil-analytical data								
pH	T.E.C. Me %	Exch. cations me %			%C	%N	%S	Truog P_2O_5 mg %
		Ca	Mg	K				
5.7	12.8	3.7	1.1	1.4	2.5	0.17	0.033	15

The experimental area had not been ploughed or treated with fertilizer for many years, and the cover was mainly sweet vernal (*Anthoxanthum odoratum*), brown-top (*Agrostis tenuis*), danthonia (*Danthonia pilosa*), fescue (*Festuca rubra fallax*), catsear (*Hypochaeris radicata*), a scattering of cocksfoot (*Dactylis glomerata*), stunted white clover (*Trifolium repens*) and suckling clover (*Trifolium dubium*).

Treatments and lay-out

Limestone was applied over the whole area at 1000 lb per acre, but in view of the naturally high phosphorus and potassium levels, no basal dressings were applied. The whole area was oversown with New Zealand pedigree white clover, Montgomery red clover and subterranean clover at 6 lb per acre. The trial was of factorial design, testing three levels of nitrogen at each of three levels of sulphur, with five blocks of replicates:

N_0 = no nitrogen
N_1 = 20 lb nitrogen per acre as nitrolime
N_2 = 60 lb ,, ,, ,, ,, ,,
S_0 = no sulphur
S_1 = 5 lb sulphur per acre as gypsum
S_2 = 15 lb ,, ,, ,, ,, ,

The size of individual plots was four square yards. The treatments were applied on October 19, 1955, the area fenced off from stock and harvested by cutting one square yard from the centre of each plot with hand shears on April 11, 1956. The herbage from each plot was separated into clover and grass (+ weeds), dried, and the replicates of each component bulked for analysis. Residual effects, which were very pronounced, were followed by taking two further cuts in the following season, on December 14, 1956, and May 6, 1957.

Rainfall

The trial was carried on for two years because of the low recoveries of sulphur in the first cut, which were due to the very dry season experienced in 1956. The rainfall was higher and more evenly distributed in the following season. In the first year the rainfall, from the time the fertilizers were applied

until harvest, 6 months later, was 14 inches. Evaporation from an open-water surface at Lincoln, which is probably less than at the experimental site, was 27 inches over the same period, and potential transpiration may be expected to have exceeded rainfall at all times except during the last six weeks. Over the winter period of 1956, from the time of harvest until the end of August, rainfall amounted to 16 inches. For the effective growing period in the second season, from the beginning of September until the final cut in early May 1957, rainfall amounted to 39 inches, and it is estimated that potential transpiration exceeded rainfall only in January and February 1957.

Analytical methods

Conventional methods were used for soil and plant analyses, as listed in previous publications [6]. Protein nitrogen was estimated by the method described by Wood and Sibly [14].

RESULTS

Yields of dry matter.

Yields in the second year (cuts 2 and 3) were double those in the first year owing to the higher rainfall (Table I). For statistical analysis, the yields for the first cut were treated separately, as grass and clover were separated from individual plots, whereas

TABLE I

Treatment	Mean yields of dry matter (lb per acre)								
	S_0			S_1			S_2		
	N_0	N_1	N_2	N_0	N_1	N_2	N_0	N_1	N_2
1st cut									
Grass	1640	1850	1690	1750	1970	2450	1790	2120	2370
Clover	30	40	20	710	480	290	640	710	410
Total	1670	1890	1710	2460	2450	2740	2430	2830	2780
2nd cut									
Grass	2890	2830	3230	3140	2760	3170	3130	3090	3090
Clover	110	30	50	1550	620	700	1290	1950	1160
Total	3000	2860	3280	4690	3380	3870	4420	5040	4250
3rd cut									
Grass	1210	1080	1150	1100	1380	1410	1300	1000	1340
Clover	100	10	10	590	540	230	980	1030	1100
Total	1310	1090	1160	1690	1920	1640	2280	2030	2440
All cuts									
Grass	5740	5760	6070	5990	6110	7030	6220	6210	6800
Clover	240	80	80	2850	1640	1220	2910	3690	2670
Total	5980	5840	6150	8840	7750	8250	9130	9900	9470

replicates were bulked before separation for the later cuts. For the first cut, the coefficients of variation were 9.5 per cent for the clover (Log. yields), 19 per cent for the grass, and 17 per cent for the grass plus clover. Owing to the very large responses of clover to sulphur, it was considered desirable to transform the clover data to logarithms for the analysis of variance, and this showed a highly significant response to sulphur, with no significant differences between S_1 and S_2, and between N_0 and N_1, but a highly significant depression from N_2. The lack of significant differences between S_1 and S_2 on clover growth in the first year, was in marked contrast to previous results [8], and as seen by the residual effects in the following two cuts, was almost certainly due to the low solubility of gypsum, because of the dry summer. Also for the first cut, grass did not respond significantly to nitrogen in the absence of sulphur, but N_2 in the presence of sulphur gave a significant increase in yield of 40 per cent. Yields of grass plus clover in the first cut showed highly significant responses to sulphur (with no significant differences between S_1 and S_2) both in the presence and absence of nitrogen, but the effect of nitrogen on total yields was not significant.

The yields for the later cuts have not been given separate statistical treatment, but the mean yields for each cut have been examined. Treatment differences for the total yields (grass plus clover) were consistent from one cut to another, and the only significant treatment responses were to sulphur, S_1 and S_2 respectively, giving yields 40 and 60 per cent higher than S_0. To determine whether the treatments had affected grass and clover differently, the data were transformed to logarithms, and the coefficient of variation (5 per cent) was very well behaved. This analysis showed that the grass-clover ratio was effectively constant from cut to cut when averaged over all treatments. Comparing the effects of different treatments on grass and clover, the difference between N_2 and N_0 was significantly different ($P = 0.01$) for grass and clover, with N_1 in a similar intermediate position for both. There was a nitrogen response on grass and a nitrogen depression on clover. The difference between S_2 and S_0 was also highly significantly different, with S_1 in a highly significantly different relation to both S_0 and S_2, for grass and clover. Grass did not respond significantly to sulphur, and for clover, S_1 gave yields significantly ($P = 0.05$) below S_2. There was no overall nitrogen-sulphur interaction, and no difference

between grass and clover in the nitrogen-sulphur interaction. In spite of this, there are strong indications from the yields of clover for individual cuts and the total yields for all cuts (Table I) that nitrogen has suppressed clover at S_0 and S_1, but has had little if any significant effect at S_2. In the analysis of the data, transformed to logarithms, this effect has apparently been masked.

The main effects of nitrogen treatments on the yields of dry matter are presented in Fig. 1a. In the first cut, the increased yields of grass from nitrogen applications were almost balanced by the suppression of clover yields. This suppression of clover continued into the second cut, but showed signs of diminishing in the third cut. The total yields for the three cuts show quite clearly that any increase in yield of grass from the use of nitrogen was offset by the depression in yield of the clover. In Fig. 1b are shown the main effects of sulphur on dry matter yields. The residual effects of sulphur on clover growth in the second and third cuts were very pronounced, supporting the suggestion that S_2 in the first year, was not significantly better than S_1, solely because dry conditions prevented the complete solution of the gypsum.

Yields of nitrogen

The total yields of nitrogen for all cuts are shown in Table II. The apparent recovery of applied nitrogen by the grass, over the

TABLE II

Yields of nitrogen (lb per acre). Total for 3 cuts									
Treatment	S_0			S_1			S_2		
	N_0	N_1	N_2	N_0	N_1	N_2	N_0	N_1	N_2
Grass	78	79	92	94	88	100	91	91	100
Clover	6	2	2	78	45	34	81	101	71
Total	84	81	94	172	133	134	172	192	171

three cuts, was very poor indeed, being virtually nil for N_1 and 9 lb for N_2 for the mean of all sulphur treatments. (The mean recoveries per acre for the first cut were 2 lb for N_1 and 8 lb for N_2). There was evidence of underground transference of nitrogen from clover to grass, because as clover growth increased at N_0 due to application of sulphur, the yield of nitrogen in the grass also increased, by 15 lb on the average of S_1 and S_2.

The main effects of nitrogen on the yields of nitrogen for the

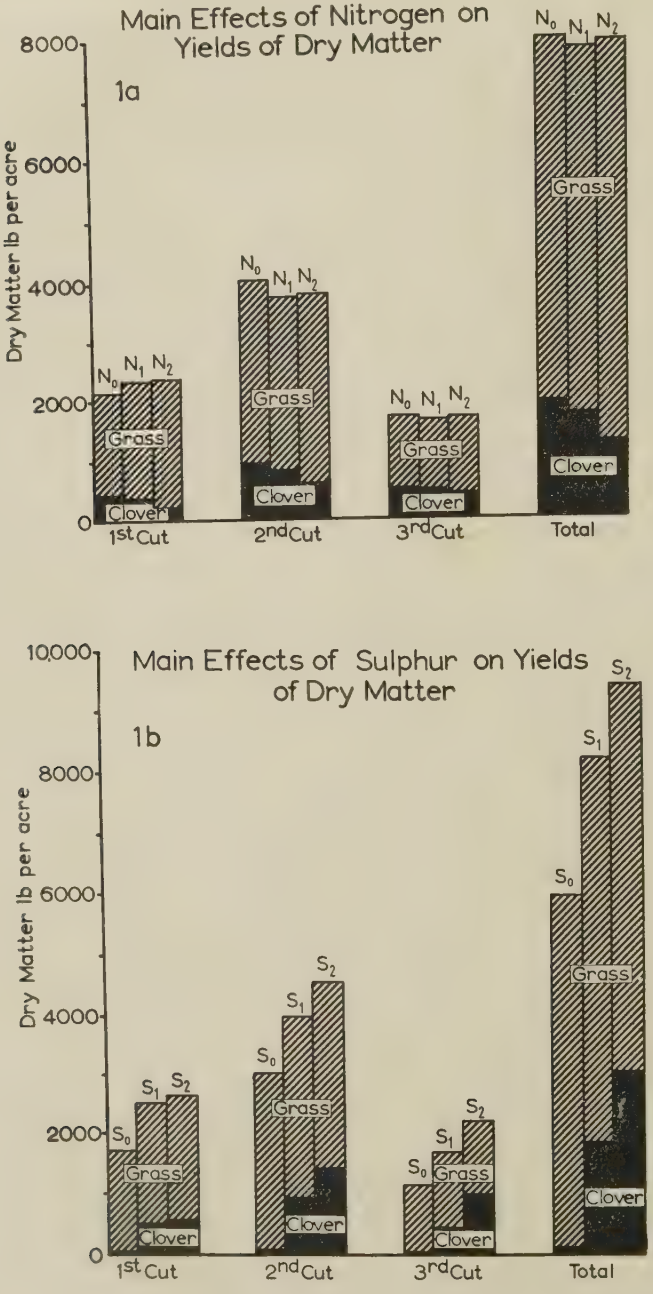

Main Effects of Nitrogen on Yields of Dry Matter
1a
Dry Matter lb per acre
8000
6000
4000
2000
0
N0 N1 N2
Grass
Clover
1st Cut
2nd Cut
3rd Cut
Total
Main Effects of Sulphur on Yields of Dry Matter
1b
10,000
S0 S1 S2

Fig. 1

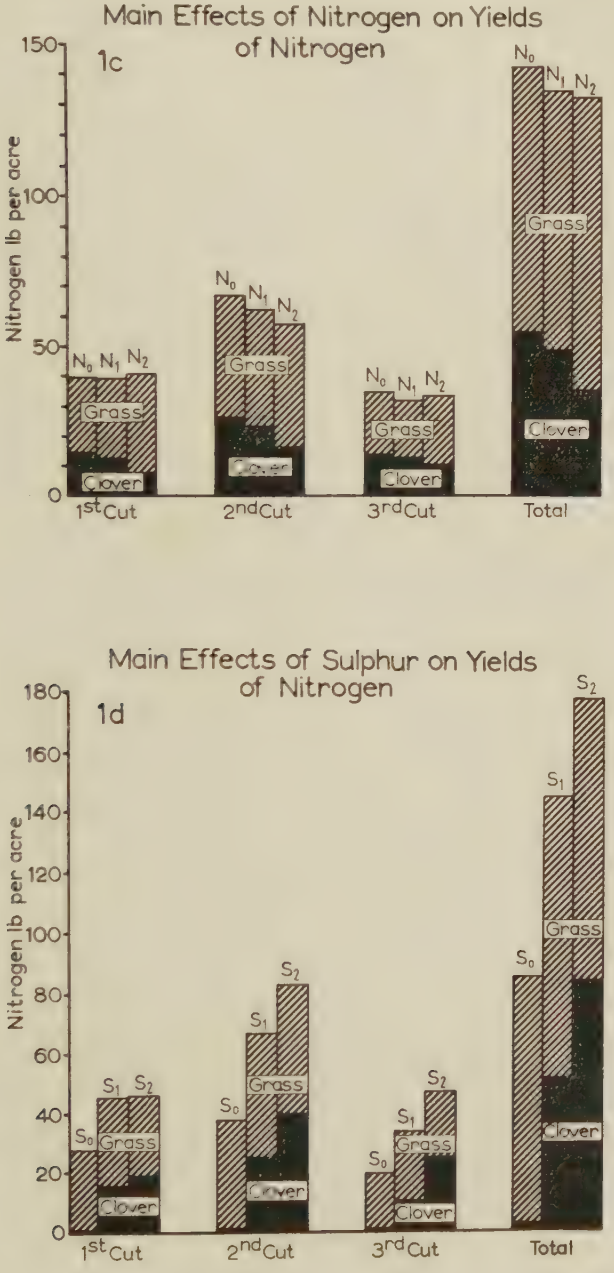

Main Effects of Nitrogen on Yields of Nitrogen
1c
Nitrogen lb per acre
150
100
50
0
N_0 N_1 N_2
Grass
Clover
1st Cut
2nd Cut
3rd Cut
Total
Main Effects of Sulphur on Yields of Nitrogen
1d
180
160
140
120
100
80
60
40
20
0
S_0 S_1 S_2

Fig. 1.

individual and total cuts are shown in Fig. 1c. This emphasises even more than the yields of dry matter, the negligible effects of fertilizer nitrogen on total yields, as clover by virtue of its higher content of nitrogen makes a relatively bigger contribution than in the case of dry matter. Indeed, the data indicate an overall depression in yields of nitrogen from the application of fertilizer nitrogen, supporting previous observations in Britain [11]. The main effects of sulphur on yields of nitrogen (Fig. 1d), show that S_2 gave double the yield of nitrogen obtained at S_0, the clover contributing almost as much as the grass.

Composition of grass and clover

For the first cut, both grass and clover were analysed for total nitrogen, non-protein nitrogen, total sulphur, sulphate sulphur, and phosphorus, as shown in Table III. The second and third cuts were

TABLE III

Composition of grass and clover, in first cut. Percent in dry matter								
Treatment	Total N	Non-protein N	Total S	SO_4-S	Total N / Total S	Protein N / Organic S	Total P
Clover							
N_0 S_0	2.65	0.83	0.11	0.006	24	17	0.31
N_0 S_1	3.25	0.85	0.17	0.005	20	15	0.24
N_0 S_2	3.23	0.87	0.22	0.006	15	11	0.22
N_1 S_0	2.67	0.75	0.12	0.006	23	18	0.36
N_1 S_1	3.23	0.89	0.17	0.007	20	15	0.23
N_1 S_2	3.24	0.62	0.19	0.005	17	14	0.22
N_2 S_0	2.98	—	0.13	0.008	23	—	—
N_2 S_1	3.15	0.87	0.17	0.006	19	14	0.23
N_2 S_2	3.46	0.83	0.16	0.006	21	17	0.22
Grass							
N_0 S_0	1.47	0.56	0.12	0.03	12	9	0.21
N_0 S_1	1.52	0.52	0.16	0.05	9	9	0.16
N_0 S_2	1.39	0.37	0.19	0.07	8	9	0.17
N_1 S_0	1.43	0.65	0.12	0.02	12	8	0.20
N_1 S_1	1.31	0.45	0.16	0.03	8	7	0.17
N_1 S_2	1.37	0.43	0.20	0.05	7	6	0.16
N_2 S_0	1.83	0.86	0.13	0.01	15	8	0.20
N_2 S_1	1.49	0.55	0.15	0.04	10	8	0.15
N_2 S_2	1.36	0.45	0.19	0.06	7	7	0.16

analysed only for total nitrogen and sulphur, for the purpose of calculating total yields of these nutrients, and are not presented.

In the absence of sulphur the total sulphur contents were extremely low and increased appreciably with application of sulphur; the levels were very similar for both grass and clover, but, as in previous years, sulphate-sulphur was higher in the grass and increased with increasing sulphur dressings, whereas sulphate-sulphur remained low throughout in the clover. The total sulphur contents were considerably lower, both in the absence and presence of sulphur, than we have yet recorded for comparable treatments.

Nitrogen treatments had little effect on the per cent total nitrogen in the clover, but sulphur treatments gave an increase mainly in protein nitrogen, as the per cent non-protein nitrogen remained almost steady. Neither nitrogen nor sulphur treatments had much effect on the per cent total nitrogen in the grass except at N_2, where because sulphur increased dry matter yields by 40 per cent, the nitrogen content fell from 1.83 to 1.36 per cent at N_2S_0 and N_2S_2 respectively, giving similar yields of nitrogen per acre (31 and 32 lb respectively). This drop in nitrogen content occurred almost entirely in the non-protein nitrogen fraction which was very high at N_2S_0. The per cent protein nitrogen in the grass remained fairly constant for all treatments (mean 0.93), and because much of the extra sulphur taken up by the grass accumulated as sulphate, the protein nitrogen organic sulphur ratio remained very steady, whereas the total nitrogen total sulphur ratio was reduced by applications of sulphur. The nitrogen sulphur ratios were considerably higher in the clover than in the grass. Anderson and Spencer [1] found total nitrogen total sulphur ratios of 30 for subterranean clover growing under conditions of acute sulphur deficiency and 7 to 9 when adequate sulphur was applied.

The phosphorus content of the herbage was adequate at all levels, justifying the omission of basal phosphate dressings. Sulphur treatments depressed phosphorus levels in both grass and clover, perhaps because they increased yields of dry matter, but it is of interest that the phosphorus content usually exceeded the sulphur content.

Uptake of sulphur

The uptake of sulphur by grass and clover for all cuts is shown in Table IV. The mean recovery over the whole period of the trial

was 94 per cent in the case of the 5-lb dressing, and 55 per cent for the 15-lb dressing of sulphur. In the first year, however, because of dry conditions only 2 lb were recovered at S_1 and 3 lb at S_2; total recoveries were higher in the second year. The percentage of sulphur

TABLE IV

Uptake of sulphur by grass and clover (1b per acre). Total for 3 cuts.

Treatments	S_0			S_1			S_2		
	N_0	N_1	N_2	N_0	N_1	N_2	N_0	N_1	N_2
Grass	8.6	8.2	8.5	9.7	9.1	1.4	10.8	10.0	12.1
Clover	0.3	0.1	0.1	4.6	3.0	2.2	5.6	7.2	5.0
Total	8.9	8.3	8.6	14.3	12.1	13.6	16.4	17.2	17.1
Mean uptake	8.6			13.3			16.9		
Mean recovery for 3 cuts				4.7 (94%)			8.3 (55%)		
Mean recovery at individual cuts				1st cut	2nd cut	3rd cut	1st cut	2nd cut	3rd cut
				2.0	1.8	0.9	3.1	3.3	1.9

taken up by the clover of the total uptake, increased from a mean of 2 per cent at S_0 and 24 per cent at S_1 to 35 per cent at S_2; in other words, under conditions of acute sulphur deficiency at S_0 grass took up 98 per cent of the total available sulphur, supporting our suggestion [5] that grass will take up most of the nitrogen and sulphur made available from the mineralisation of organic matter. This figure of 98 per cent should be compared with the estimate of 95 per cent uptake of combined nitrogen by grass when labelled nitrogen was applied to a grass-clover association [9]. A large fraction of the sulphur recovered in the grass (almost a third of the total uptake at S_2) was in the inorganic form at the first cut (Table III). It is significant that this accumulation of SO_4-S took place even when combined nitrogen was applied, suggesting that most of the nitrogen was simply not taken up by the grass, substantiated of course by the recovery data for nitrogen. A shortage of sulphur at N_2S_0 might conceivably have limited uptake of nitrogen by the grass, but at N_2S_2 there was clearly adequate SO_4-S to have permitted a much greater uptake of nitrogen, whereas in actual fact yields of nitrogen were simular at N_2S_0 and N_2S_2 at the first cut.

DISCUSSION

Levels of gypsum, supplying 0, 5 and 15 lb of sulphur per acre were chosen in this trial on the evidence of previous results [8] to give responses to sulphur on the steep part of the response curve. Nitrogen was applied at 0, 20 and 60 lb per acre, with the expectation that by stimulating the grass, it would cause severe competition for sulphur and lead to clover suppression at the 0- and 5-lb rates, whereas the 15-lb rate should have supplied enough to meet the extra needs of the grass and still leave excess sulphur to promote nitrogen fixation by clovers. Owing to abnormally dry conditions in the first year, differences between the 5- and 15-lb dressings of sulphur were not significant, but the heaviest dressing of nitrogen increased grass yields and depressed clover yields significantly but had no significant effect on total yields of grass plus clover.

It seemed reasonable to assume that the heavy dressing of gypsum was less effective than expected because solution was restricted by the dry conditions and the experiment was allowed to proceed without further treatment for another year. As shown in Table I and Figs. 1b and 1d, there were marked residual effects from sulphur, and over the two seasons significant differences were found between S_1 and S_2 on the growth of clover. Examination of the total yields of dry matter for all three cuts in Table I, shows that at S_0 and S_1 nitrogen suppressed clover appreciably, but had little if any such effect at S_2.

More sulphur was recovered in the second year than in the first. Clearly much of the sulphur was held over in the soil as gypsum, or having been brought into solution, was not completely leached below root depth, or was adsorbed by the soil colloids [3]. It may also have been retained in the root systems of the pasture species, or immobilised by soil organisms. As the mechanism of sulphur retention by the soil will be the subject of later publications it is not intended to discuss the matter at this stage.

It is difficult to account for the poor recovery of nitrogen by the grass, as in previous experiments [10] some 50–65 per cent of the nitrogen applied was recovered in the grass. This may have been due to the inferior species of which the sward was largely composed, as Wagner [12] has shown that Brome grass (*Bromus inermis*)

recovered much less applied nitrogen than cocksfoot or tall fescue (*Festuca arundinacea*). Alternatively the nitrogen may have been immobilised by micro-organisms because of the fairly high C : N ratio of 15, or it may have been denitrified or leached. Although we have recently reported pot trials where 30 per cent of the applied labelled nitrogen was lost by denitrification [9], soil conditions here were not those normally associated with high losses in this way. It is difficult to believe that nitrogen was lost by leaching under the same conditions that limited the solution of gypsum. It is assumed, based on earlier work [9] with N^{15}, that most of the nitrogen in the clover was fixed symbiotically. If, however, the particular climate and species favoured the clover in competition with the grass for combined nitrogen, then some of this nitrogen may have been recovered by the clover at N_1 and N_2. This would still not account for a high proportion of the nitrogen applied at the N_2 rates in the first cut, and there was no further recovery of nitrogen in the second year.

Although, owing to the poor recovery of nitrogen and sulphur in the first season, the competition between grass and clover for sulphur was restricted to a much narrower range than planned, the results showed conclusively that grass can compete very effectively for sulphur under conditions of acute deficiency. The suppression of clover may have been intensified had the recovery of nitrogen been higher. Suppression of clover may be minimised by applying adequate sulphur when fertilizer nitrogen is used, in the same way as applications of potassium and phosphorus will help to maintain the contribution of clover in soils deficient in these elements, at least until light or moisture become the limiting factors.

Nitrogen fertilizers not containing sulphur would be virtually useless in this area for application to pastures, but sulphate of ammonia by virtue of its large excess of sulphur over nitrogen in terms of plant requirements, would be expected to stimulate both grass and clover, if the usual precautions were taken against its effect on soil acidity. A comparison of the total yields of nitrogen and sulphur for all treatments in Tables II and IV shows that the herbage as a whole contained about ten times more nitrogen than sulphur, but as seen in Table III, the N : S ratio in the clover was almost twice that in the grass. In other words, for a given amount of sulphur, clover may fix considerably more nitrogen symbioti-

cally than grass can take up in the mineral form, and this is due largely to the luxury consumption by grass of sulphate.

The general picture provided by the analytical data on inorganic and total sulphur, protein and non-protein nitrogen, conforms with the data of others (*e.g.* Anderson and Spencer [1], Wood and Barrien [13]). The percent protein nitrogen in the grass at the first cut remained remarkably steady for the various levels of nitrogen and sulphur, and increases in yield of protein nitrogen were a reflection of increased yields of dry matter rather than increased contents of protein nitrogen. Anderson and Spencer [1] in a trial on oats with basal nitrogen, increased the yield of dry matter appreciably on application of sulphur as manganese sulphate; the per cent total nitrogen fell from 3.28 to 1.93 per cent, total yields of nitrogen remained constant and the per cent protein nitrogen increased from 0.88 to only 1.08. Potassium sulphate applied only 20 days before harvest however, and accordingly giving a much smaller increase in yield of dry matter, increased the protein nitrogen to 1.66 per cent. It is possible that photosynthesis may be restricted in grass if the content of protein nitrogen remains much below 1 per cent, where this is caused, say, by sulphur deficiency. Correction of the sulphur deficiency will increase the formation of protein nitrogen, and if adequate time is given for this to influence photosynthesis, it may be reflected in increased yields of dry matter rather than in higher contents of protein. With still heavier applications of nitrogen and sulphur, the rate of photosynthesis would become limiting eventually and the protein content would rise.

SUMMARY

A 3×3 factorial experiment with nitrogen and sulphur fertilizers was carried out on a grass-clover association in an area where there is little if any cyclic return of sulphur. In the absence of sulphur, grass took up 98 per cent of the total uptake of sulphur, and nitrogen fixation by associated clover was negligible. In the absence of fertilizer nitrogen, dressings of sulphur as gypsum stimulated clover growth; at a low rate of application of sulphur, nitrogen fertilizer increased grass growth and clover was suppressed. This suppression was largely overcome with a higher dressing of sulphur. Sulphur therefore must be added to the group of elements (phosphorus, nitrogen and potassium) for which grass may compete intensely when grown with clover and when nitrogen fertilizers are applied to a grass-clover association

adequate supplies of sulphur must be assured in order to minimise suppression of clover. Sulphate of ammonia may stimulate clover growth under these conditions. Solubility of gypsum was low in a dry season and residual effects were pronounced.

ACKNOWLEDGEMENTS

We wish to thank Mr. C. J. Cran for his sustained interest and permission to experiment on his farm. Also Miss P. Hartley and various students for technical assistance. Mr. R. C. Blackmore for the figures Mr. G. Wright for the statistical analyses, and the Department of Scientific and Industrial Research for a grant covering the cost of this work.

Received September 6, 1957.

REFERENCES

1 Anderson, A. J. and Spencer, D., Sulphur in nitrogen metabolism of legumes and non-legumes. Australian J. Sci. Research **3**, 431–449 (1950).
2 Blaser, R. E. and Brady, N. C., Nutrient competition in plant associations. Agron. J. **42**, 128–135 (1950).
3 Ensminger, L. E., Some factors affecting the adsorption of sulphate by Alabama soils. Soil Sci. Soc. Am. Proc. **18**, 259–264 (1954).
4 Trumble, H. C. and Shapter, R. E., The influence of nitrogen and phosporus treatment on the yield and chemical composition of Wimmera ryegrass and subterranean clover, grown separately and in association. Australia C.S.I.R. Bull. **105**, 25–36 (1937).
5 Walker, T. W., The sulphur cycle in grassland soils. J. Brit. Grassland Soc. **9**, 10–18 (1957).
6 Walker, T. W., Adams, A. F. R. and Orchiston, H. D., Some effects of sulphur and phosphorus on the yield and composition of rape. New Zealand J. Sci. Technol. **36a**, 103–110 (1954).
7 Walker, T. W., Adams, A. F. R. and Orchiston, H. D., The effects and interactions of sulphur, phosphorus and molybdenum on the growth and composition of clovers. New Zealand J. Sci. Technol. **36**a, 470–482 (1955).
8 Walker, T. W., Adams A. F. R. and Orchiston, H. D., The effect of levels of calcium sulphate on the yield and composition of a grass and clover pasture. Plant and Soil **7**, 290–300 (1956).
9 Walker, T. W., Adams, A. F. R. and Orchiston, H. D., The fate of labelled nitrate and ammonium nitrogen when applied to grass and clover grown separately and together. Soil Sci. **81**, 339–351 (1956).
10 Walker, T. W., Orchiston, H. D. and Adams, A. F. R., The nitrogen economy of grass-legume associations. J. Brit. Grassland Soc. **9**, 249–274 (1954).
11 Walker, T. W., Edwards, G. H. A. *et al.*, The use of fertilizers on herbage cut for conservation. Part I. J. Brit. Grassland Soc. **7**, 107–130, (1952).
12 Wagner, R. E., Legume nitrogen versus fertilizer nitrogen in protein production from forage. Agron. J. **46**, 233–237 (1954).
13 Wood, J. G. and Barrien, B. S., Studies on the sulphur metabolism of plants. New Phytol. **38**, 125–149 (1939).
14 Wood, J. G. and Sibly, P. M., Carbonic anhydrase activity in plants in relation to zinc content. Australian J. Sci. Research **5**, 244–255 (1952).

Plant and Soil IX, no 4 July 1958

ÉTUDE DE LA DÉCOMPOSITION DE LA MATIÈRE ORGANIQUE DANS LE SOL, AU MOYEN DE CARBONE RADIOACTIF *

I. CINÉTIQUE DE L'OXYDATION EN CO_2 DE DIVERS SUBSTRATS RADIOACTIFS

par PAUL SIMONART et JACQUES MAYAUDON

Centre de Microbiologie du Sol et de l'Eau, Université de Louvain, Heverlé, Belgique

I. INTRODUCTION

La complexité des processus microbiens du sol autant que la multiplicité et la diversité des substrats attaqués rendent particulièrement difficile l'étude des mécanismes de transformations des divers groupes de substances qui se décomposent dans le sol. C'est pourquoi, il s'indiquait, pour chercher à déchiffrer certaines phases de la décomposition de la matière organique dans le sol, de recourir à l'emploi de substrats à carbone radioactif C^{14}. Il est ainsi possible de suivre "à la trace" la destinée de tel ou tel composé, ce qui est exclu par les méthodes habituelles de chimie.

Une communication au Congrès de la Science du Sol [5] résumait les premiers résultats obtenus dans ce domaine. Cette note-ci présente les détails expérimentaux et les données chiffrées relatives à la cinétique de la décomposition dans le sol de matériel végétal et de plusieurs substrats organiques déterminés.

II. MÉTHODES EXPÉRIMENTALES

A. *Production de matériel végétal radioactif*

On prend environ 2 grammes de plantes adultes de ray-grass,

* Travail effectué sous les auspices de l'Institut pour l'Encouragement de la Recherche Scientifique dans l'Industrie et l'Agriculture (I.R.S.I.A.).

munies de leurs racines, et on les place dans un cylindre de verre de 20 × 4 cm, terminé de part et d'autre par un robinet (fig. 1); les racines baignent dans 5 ml de solution préconisée par Hoagland et Arnon [3].

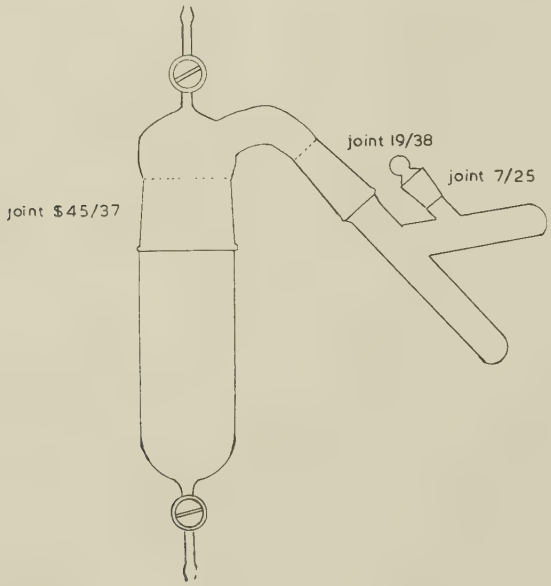


Figure 1.

Dans une des deux branches du tube en Y, qui est relié au cylindre, on introduit 9 mg $BaC^{14}O_3$ (1 mc d'activité*), et dans l'autre branche on place 3 ml d'acide lactique à 80%. On aspire par le cylindre de l'air, exempt de CO_2, puis on ferme les robinets et, par une rotation du tube en Y, on provoque la génération du $C^{14}O_2$ dans l'appareil par mise en contact de l'acide lactique avec le $BaC^{14}O_3$. On place le dispositif au voisinage de deux lampes de 250 W, et au moyen d'une ventilation d'air froid, on maintient à la température de 25°C. Après 24 heures, on recueille la partie aérienne des herbes. La synthèse chlorophyllienne de plantes adultes assure la radioactivité de tous les constituants étudiés: tissus de soutien, substances solubles et pigments.

* A.E.R.E., Harwell, Angleterre.

B. *Fractionnement du matériel végétal*:

Pour séparer les substances solubles, les substances du type hemicellulose et la cellulose avec la lignine, on opère de la façon décrite ci-dessous:

Fraction des substances solubles. On plonge les herbes radioactives dans 100 ml d'alcool à 80% bouillant et on chauffe à reflux pendant 10 minutes. On recueille l'extrait alcoolique et on renouvelle la décoction dans les mêmes conditions. On extrait ensuite les plantes à l'alcool à 20% bouillant. On réunit les extraits alcooliques, on les évapore sous vide jusqu'à un faible volume et on porte la solution à neutralité à l'aide de NaOH 0,01*N*. Ceci constitue la fraction soluble.

Fraction des hemicelluloses. Après extraction de la fraction soluble, on lave à l'éther le résidu qui contient les tissus de soutien et on le sèche à l'air. Puis on laisse au contact de 5 ml de NaOH *N*, pendant 24 heures; on répète une seconde fois ce traitement. On réunit ces deux extraits alcalins, on acidifie à pH = 4,0 — 4,5 au moyen d'acide acétique et on en précipite les hemicelluloses par addition d'un égal volume d'alcool à 95%. Le précipité est lavé à l'eau et à l'alcool.

Fraction cellulosique. Le résidu des extractions précédentes contient approximativement 80% de cellulose et 20% de substances non hydrolysables (lignine). Comme la séparation l'un de l'autre, de ces deux constituants, par les méthodes classiques, va de pair avec une altération chimique, on préfère utiliser cette fraction telle quelle sans purification plus poussée.

C. *Mesure de la radioactivité dans le matériel végétal:*

A titre d'exemple, voici la répartition de la radioactivité obtenue pour un gramme de ray-grass laissé en atmosphère de $C^{14}O_2$ selon la méthode qui vient d'être décrite:

Matériel	Radioactivité	mg C
	millions coups/min	
Substances solubles	42,0	40,0
Hemicelluloses	0,4	8,4
Cellulose + lignine	5,9	20,8

Les chiffres du précédent tableau ont été obtenus de la façon suivante: Pour le dosage du carbone on fait la combustion, suivant

la méthode de Van Slyke-Folch [6], d'une partie aliquote de chacune des fractions organiques séparées. La quantité de carbone est obtenue par multiplication du poids de $BaCO_3$ recueilli, par le facteur 0.06085. Quant à la radioactivité, on la mesure au compteur Geiger-Müller, sur un échantillon de $BaCO_3$ étalé sur une plaque d'aluminium [2]; on l'exprime en coups par minute (un coup = 23.0 désintégrations).

D. *Répartition de la radioactivité dans les fractions organiques végétales:*

Fraction soluble. Dans cette fraction, on sépare l'un de l'autre, selon les méthodes décrites ci-dessous, les pigments et les cires (substances solubles dans l'éther), les sucres et les acides aminés (substances solubles dialysables) et les substances de nature protéique (substances solubles non dialysables).

- substances solubles dans l'éther:
  Une fraction aliquote de la fraction soluble est évaporée jusqu'à sec *in vacuo* et le résidu d'évaporation repris à l'éther sulfurique. Les extraits d'éther sulfurique sont réunis et lavés plusieurs fois à l'eau distillée puis séchés. Cet extrait après évaporation de l'éther est repris dans 2 cc d'acétone. Les pigments ainsi solubilisées sont séparés par chromatographie de partage sur papier Whatman 3 MM, en utilisant comme solvant, d'une part, l'éther de pétrole (pt éb.: 60°)/propanol dans les proportions 100/1 (v/v), d'autre part, l'éther de pétrole (pt éb.: 60°)/chloroforme dans les proportions 3/1 (v/v) [4]. On donnera ultérieurement les détails opératoires concernant cette séparation.
- substances solubles dialysables et non dialysables:
  Une partie aliquote de la fraction soluble est passée à travers un entonnoir en verre fritté IG3. On aide la filtration par addition de celite analytique (Light). On introduit le filtrat dans un sac de cellophane et on dialyse sous faible agitation pendant 48 heures, contre eau distillée à 5°C de température. Le contenu du sac contient les substances de nature protéique, tandis que le dialysat après évaporation sous un faible volume est chromatographié dans le butanol/acide propionique/eau et phénol/eau [1]. Les constituants ainsi séparés sont localisés par autoradiographie et la radioactivité déterminée par application d'un tube Geiger sur le papier [1].

La répartition de la radioactivité dans la fraction soluble s'établit comme suit:

Matériel	Radioactivité	mg C
	millions coups/min	
Substances solubles dans l'éther	1,5	10,0
Substances dialysables solubles dans l'eau	36,1	17,4
Substances non dialysables solubles dans l'eau	3,4	12,6

On décèle les pourcentages de radioactivité suivants pour les principaux constituants séparés par chromatographie:

- substances solubles dans l'éther
  - chlorophylles a et b: 11%
  - carotène et xanthophylle: 31%
  - résidu (cires): 58%
- substances dialysables solubles dans l'eau:
  - glucose: 71%
  - acide glutamique: 10%
  - alanine: 8%
  - acide malique: 3,5%
  - acide citrique: 1%
  - esters phosphoriques: 1%
- fraction des hemicelluloses:
  - par hydrolyse d'une partie aliquote par HCl *N* pendant 6 heures à 100°C, on décèle par chromatographie l'acide galacturonique, un hexose, le xylose et l'arabinose dont les radioactivités représentent respectivement 14%, 33%, 30% et 21% de l'activité totale de la fraction.
- fraction cellulose + lignine.
  - sur une partie aliquote, on a opéré une digestion de 4 heures dans H_2SO_4 à 70%, suivi d'une hydrolyse de 6 heures dans H_2SO_4 2*N*. On a identifié dans cette fraction le glucose (78%), le xylose (10%), l'arabinose (7%); le reste (5%) est constitué par des acides aminés. Le résidu d'hydrolyse de caractère ligneux représente 7% de l'activité totale de la fraction.

E. *Décomposition des substrats organiques:*

On soumet à la décomposition dans le sol les fractions dont il vient d'être question, de même que du glucose radioactif * ou encore le ray-grass radioactif produit selon A. On opère de la façon suivante:

Dans des erlenmeyers, à col rôdé, de 50 ml de capacité, on place une quantité de sol – correspondant à 4 g de poids sec – provenant de l'horizon A_0 d'une prairie et passé au tamis de 9 mailles par pouce. On y ajoute une quantité de substrat radioactif correspondant à 10 mg C et la quantité d'eau nécessaire à obtenir 35% d'humidité. On bouche l'erlenmeyer au moyen d'un bouchon rôdé

* Commissariat à l'Energie Atomique, France.

pourvu de deux tubulures et on maintient à la température du laboratoire pendant toute la durée de l'expérience.

F. *Mesure du $C^{14}O_2$ formé:*

Une des deux tubulures de l'erlenmeyer sert à l'alimentation en air, débarassé de CO_2, la seconde, qui sert à l'évacuation de l'air chargé du CO_2 produit, est reliée à une trappe de CO_2 constituée d'un barboteur contenant 10 ml NaOH *N*. Par une aspiration douce et continue, un faible courant d'air traverse le dispositif pendant une période de deux mois. L'anhydride carbonique recueilli dans la trappe est précipité sous forme de $BaCO_3$ par addition de 1 ml d'une solution à 30% de $BaCl_2$ et 1 ml d'une solution à 35% de NH_4NO_3. On recueille sur un filtre de verre Jena G3, le précipité de $BaC^{14}O_3$ qu'on lave d'abord à l'eau chaude, ensuite à l'alcool et qu'on séche enfin à 100°C pendant trois heures. On place une fraction aliquote de $BaC^{14}O_3$ sur un disque d'aluminium et on en détermine la radioactivité au moyen d'un tube Geiger [2].

III. CINÉTIQUE DE LA DÉCOMPOSITION DE SUBSTRATS RADIOACTIFS DANS LE SOL

En appliquant les méthodes qui viennent d'être décrites, on a pu mesurer en fonction du temps, la quantité de CO_2 formé aux dépens de chacun des substrats employés. Le tableau I donne les valeurs

TABLEAU I

Dégagement de $C^{14}O_2$ aux dépens de divers substrats

Substrat	Glucose		Cellulose		Hemi-cellulose		Extr. soluble		Ray-grass	
Val. init.	344.097 c/min		331.600 c/min		331.150 c/min		685.150 c/min		770.675 c/min	
J	c/min	%	c/min	%	c/min	%	c/min	%	c/min	%
3	25.630*	7,45*	15.200	4,59	16.650	5,03	98.935	14,44	187.576	24,10
10	237.380	69,00	60.601	18,31	166.315	50,25	261.678	38,20	110.760	14,38
14	14.420	4,19	37.207	11,24	22.776	6,88	54.200	7,91	65.858	8,55
21	17.118	4,98	62.650	18,93	22.752	6,87	58.660	8,56	95.174	12,36
33	9.216	2,68	75.154	22,70	20.160	6,09	43.520	6,35	64.640	8,39
42	2.294	0,66	25.420	7,68	7.970	2,40	15.359	2,24	44.894	5,83
51			8.086	2,44	7.393	2,23	12.378	1,80	31.328	4,05
60			4.928	1,49	3.520	1,06	5.312	0,77	17.408	2,26
Total	305.058	88,96	289.246	87,38	267.536	80,81	550.042	80,27	617.638	80,00

* Les quantités de $C^{14}O_2$ sont exprimées en coups par minute (c/min); les % sont exprimés par rapport à la valeur initiale (val. init.) de la radioactivité du substrat.

ainsi obtenues, que la figure 2 reproduit graphiquement. Ces courbes de dégagement de $C^{14}O_2$ font ressortir une différence entre les divers substrats dans la rapidité de décomposition en CO_2. En effet, on constate qu'en 10 jours 75% du glucose sont décomposés en CO_2, tandis que pendant cette même période, il n'y a que 22% de la cellulose qui se retrouve sous forme de CO_2. Quant à la fraction des substances solubles et aux hemicelluloses, 50% en sont

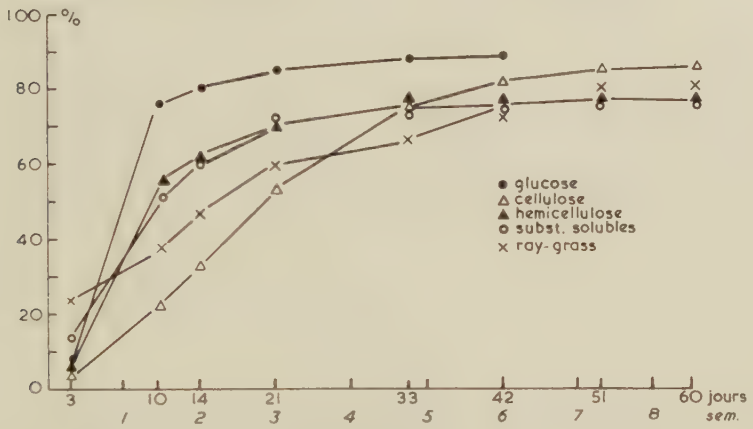


Figure 2. Courbes de décomposition en $C^{14}O_2$, de substrats radioactifs.

oxydés en CO_2 en 10 jours; on remarquera d'ailleurs que les courbes de dégagement de CO_2, pour ces deux substrats sont pratiquement identiques.

Pour ce qui est du ray-grass tel quel, la courbe de dégagement de CO_2 est, pendant quatre semaines environ, intermédiaire entre celle des substances solubles et des hemicelluloses et celle de la cellulose. A partir de cette période, elle reste en deça de la courbe de cellulose, problablement par suite d'une attaque moins aisée de constituants cellulaires que de substances chimiques isolées. Néanmoins, après deux mois, la courbe de la fraction cellulosique et celle du ray-grass se rejoignent.

Bien que d'un substrat à l'autre la courbe de dégagement de CO_2 soit différente, on remarque qu'aucun des substrats n'est complètement oxydé en CO_2: 11% du glucose, 15% des hemicelluloses et 21% de la fraction cellulosique ou encore du ray-grass tel quel, ne

se retrouvent pas sous forme de CO_2 après deux mois, alors que le dégagement de CO_2 est devenu négligeable.

Après huit semaines de décomposition dans le sol de divers substrats radioactifs, on analyse le sol pour y rechercher dans quelle proportion s'y retrouvent des substances organiques radioactives. Pour cela on sépare les substances organiques du sol suivant un mode de fractionnement qui correspond à celui du matériel végétal radioactif tel qu'il est décrit plus haut (II, B).

Trois fractions sont ainsi isolées: les substances solubles, les substances solubles dans NaOH et précipitables en milieu alcoolique à pH = 4,0 — 4,5. Sans lavage préliminaire à l'eau, les substances sont lavées à l'alcool à 94%. Cette fraction réunit l'α- et le β-humus; les acides hymatomélaniques sont partiellement solubilisés par ce traitement. La troisième fraction représente les substances insolubles dans NaOH (humine). Les acides fulviques n'ont pas été dosés.

De l'examen du tableau II, on peut observer que la radioactivité se trouve répartie entre les fractions organiques du sol dans des

TABLEAU II

Radioactivité de trois fractions organiques du sol obtenues après huit semaines aux dépens de substrats radioactifs

Substrat	Glucose		Cellulose		Hemi-cellulose		Extr. soluble		Ray-grass	
Radioactivité initiale *	344.097 c/min		331.600 c/min		331.150 c/min		685.150 c/min		770.675 c/min	
	c/min	%**	c/min	%	c/min	%	c/min	%	c/min	%
Substances solubles	5.600	1,56	10.300	3,12	7.300	2,21	25.600	3,76	10.300	4,10
α- et β-humus	6.034	1,76	11.140	3,15	13.892	4,21	27.799	4,10	11.140	4,54
humine	14.690	4,33	24.734	7,28	24.442	7,12	57.131	8,39	24.734	11,44
Total		7,65		13,55		13,54		16,25		20,08

* Exprimée en nombre de coups par minute

** Exprimé par rapport à la radioactivité initiale

proportions peu différentes quel que soit le substrat radioactif introduit au début de l'expérience. D'autre part, l'humine possède à elle seule une radioactivité qui est du même ordre de grandeur que la somme des deux autres fractions considérées.

Une prochaine étude fractionnera davantage la matière organique radioactive afin de mieux préciser les groupes de substances produites aux dépens d'un substrat déterminé.

IV. CONCLUSIONS

Par l'emploi de substrats radioactifs, il a été possible de montrer que le glucose, le ray-grass tel quel, la fraction soluble, les hemicelluloses et la fraction cellulosique du ray-grass ne sont pas, dans les conditions expérimentales appliquées, entièrement transformés en CO_2 dans le sol après une période de deux mois.

La décomposition du glucose est plus rapide que celle des hemicelluloses et celle-ci est plus rapide que celle de la fraction cellulosique. Pour le glucose et pour la fraction cellulosique, 11% et 15% respectivement ne se retrouvent pas sous forme de $C^{14}O_2$ tandis que pour la fraction soluble, pour les hemicelluloses et pour le ray-grass tel quel, c'est près de 20% qui ne sont pas oxydés en CO_2.

La décomposition de chacun de ces substrats radioactifs donne encore lieu à la production de produits organiques radioactifs que l'on trouve dans la fraction des substances solubles, dans la fraction groupant l'α- et le β-humus et dans l'humine. La radioactivité de cette dernière fraction est du même ordre de grandeur que la somme des deux autres fractions.

Reçu le 12 septembre 1957.

BIBLIOGRAPHIE

1 Benson, A. A., Bassham, J. A., Calvin, M., Goodale, T. C., Haas, V. and Stepka, W., The path of carbon in photosynthesis. I. – Paper chromatography and radioautography of the products. J. Am. Chem. Soc. **72**, 1710–1718, (1950).

2 Calvin, M., Heidelberger C., Reid, J., Tolbert, B. and Yankwich, P., Isotopic Carbon. J. Wiley & Sons, New York (1949).

3 Hoagland, D. R. and Arnon, D. I., Solution experiments with higher plants. California Agr. Expt. Sta. Circ. 347, December 1938.

4 Lind, E. F., Lane, H. C. and Gleason, L. S., Partial separation of the plastic pigments by paper chromatography. Plant Physiol. **28**, 325-328 (1953).

5 Simonart, P. et Mayaudon, J., Étude de la décomposition de la matière organique dans le sol au moyen de carbone radioactif. Congr. Intern. de la Science du Sol, Paris 1956, C.R. du 6e Congrès, (sous presse).

6 Van Slyke, D. D. and Folch, J., Manometric carbon determination, J. Biol. Chem. **136**, 509-541 (1940).

Plant and Soil IX, no 4 July 1958

ÉTUDE DE LA DÉCOMPOSITION DE LA MATIÈRE ORGANIQUE DANS LE SOL AU MOYEN DE CARBONE RADIOACTIF *

II. DÉCOMPOSITION DU GLUCOSE RADIOACTIF DANS LE SOL

A. RÉPARTITION DE LA RADIOACTIVITÉ DANS LES FRACTIONS HUMIQUES DU SOL

par JACQUES MAYAUDON et PAUL SIMONART

Centre de Microbiologie du Sol et de l'Eau, Université de Louvain, Hévérlé, Belgique

I. INTRODUCTION

Dans une publication précédente [2], on a montré que, dans le sol et dans les conditions expérimentales appliquées, les substrats organiques étudiés ne sont pas totalement oxydés en CO_2, mais donnent lieu à la production d'humus et d'humine. Il s'indique d'étudier plus en détail la répartition de la radioactivité des diverses fractions de ces complexes organiques. C'est là l'objet de la présente note, qui porte exclusivement sur la décomposition du glucose uniformément radioactif.**

II. MÉTHODES EXPÉRIMENTALES

On a effectué le fractionnement de la matière organique du sol selon la méthode détaillée ci-dessous en désignant les fractions selon la nomenclature employée par Waksman [4] et Wittich [5].

A. *Fractionnement de la matière organique du sol*

A une quantité de sol correspondante à 4 g de poids sec, on ajoute 20 ml d'alcool à 80%, on chauffe à reflux pendant 15 minutes et on sépare l'extrait alcoolique par centrifugation à 3.000

* Travail effectué sous les auspices de l'Institut pour l'Encouragement de la Recherche Scientifique dans l'Industrie et l'Agriculture (I.R.S.I.A.).

** Commissariat à l'Energie Atomique, France.

t min pendant 5 minutes; on met le précipité de centrifugation en suspension dans 10 ml d'alcool à 80°$_0$, et on centrifuge à nouveau. On extrait ensuite à l'alcool à 20°$_0$ en opérant comme pour l'alcool à 80%. On réunit les extraits alcooliques et on les filtre au travers d'un filtre de verre fritté. L'ensemble de ces extraits alcooliques constitue la *fraction soluble*. Elle contient dans le cas présent le substrat primitivement introduit et non encore humifié.

Le sol contient les fractions organiques insolubles dans l'eau. Ces fractions seront encore appelées les *fractions humiques*. On opère l'extraction avec NaOH 0,1 *N* en atmosphère d'azote pour éviter les dangers d'oxydation de la matière lignifiée en présence d'oxygène [1]. On chauffe doucement jusqu'à 90°C pendant 45 minutes. On laisse ensuite reposer pendant 3 heures. On centrifuge comme précédemment; on lave le sol avec 10 ml NaOH 0,1*N* et 20 ml d'eau distillée; on sépare du sol par centrifugation les solutions alcalines et aqueuses. On réunit les eaux de lavage à l'extrait alcalin. On clarifie l'extrait brut par addition de celite analytique et on fait une nouvelle centrifugation. Cet extrait alcalin contient l'α-humus (ou les acides humiques), les substances hymatomélaniques, les acides fulviques et le β-humus.

Pour séparer ces fractions l'une de l'autre, on opère comme suit: On acidifie l'extrait alcalin au moyen d'HCl *N* jusqu'à pH = 2,0. On recueille par centrifugation le précipité brun qui se forme, on le met en suspension dans 5 ml d'HCl 0,01*N* et on centrifuge à nouveau; on remet le précipité en suspension dans 5 ml d'alcool à 94% et on centrifuge; on répète cette opération et on réunit les extraits alcooliques, qui, après évaporation de l'alcool, donnent une fraction rouge brun composée *d'acides hymatomélaniques*. Le résidu des extractions alcooliques est brun foncé et constitue l'*α-humus*.

On réunit les extraits acides et on les réduit sous vide à 5 ml; on centrifuge pour éliminer le léger trouble qui pourrait s'être formé; on ajuste le pH à 4,8, et on ajoute à la solution son volume d'alcool à 94–95%. Après repos de 15 minutes, on sépare par centrifugation les substances colloïdales brun clair, qui ont floculé et qui constituent le *β-humus*. Le liquide surnageant, qui est de couleur jaune, contient les *acides fulviques*.

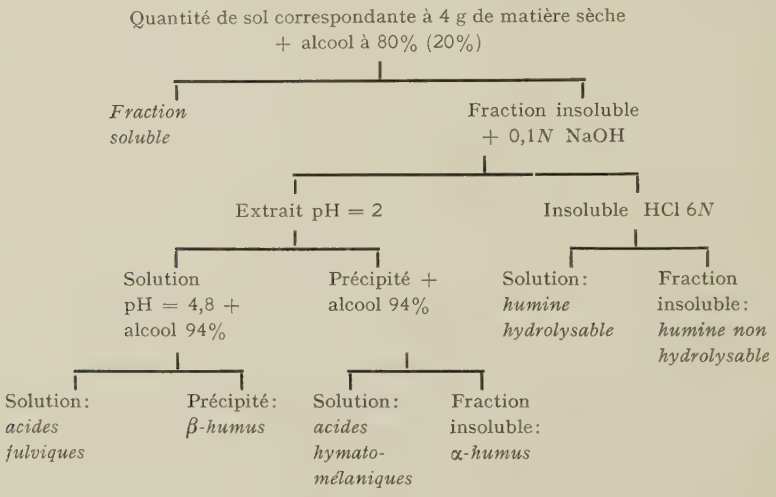
En plus des fractions dont il vient d'être question, le sol, après lavage à l'alcool et l'éther, contient encore des matières organiques insolubles, encore appelées *humine*. On trouve dans cette fraction

les scléroprotéines, les protéines rendues insolubles par dénaturation, les celluloses, les lignines, etc. On peut la partager en fractions hydrolysable et non hydrolysable. Pour obtenir la fraction hydrolysable de l'humine on procède à l'hydrolyse, dans 5 ml HCl 6*N* de 500 mg de sol sec, en tube scellé à 100° pendant 24 heures.

Le fractionnement qui vient d'être décrit, est schématisé dans le tableau I.

TABLEAU I

Schema de fractionnement de la matière organique du sol



B. *Détermination du carbone*

Toutes les fractions étudiées sont uniformément portées au volume de 8 ml, l'α-humus, le β-humus et les acides hymatomélaniques étant solubilisés dans NaOH 0,1*N*. On prend 2 ml de chacune de ces solutions, on y ajoute 2 ml d'HCl 0,1*N* et on évapore à sec sous pression réduite pour éliminer toute trace de carbonate.

On détermine la teneur en carbone de ces résidus d'évaporation, par combustion humide selon Van Slyke-Folch [3]. La teneur en carbone de l'humine hydrolysable s'évalue sur la totalité de l'hydrolysat chlorhydrique auquel on ajoute l'eau distillée ayant servi à laver le résidu d'hydrolyse. On évapore l'hydrolysat *in vacuo* et on renouvelle plusieurs fois l'évaporation après addition d'eau distillée pour éliminer complètement l'excès d'HCl. La teneur en carbone de l'humine non hydrolysée se détermine sur le résidu d'hydrolyse.

III. RÉSULTATS ET DISCUSSION

Il est intéressant de rappeler que, au cours de la décomposition du glucose dans le sol, une partie du carbone se retrouve sous forme de composés organiques et notamment de substances humiques [2]. Les résultats qui suivent confirment d'ailleurs cette observation.

Dans une seconde série d'expériences au moyen de glucose radioactif, on a, pendant soixante jours, périodiquement déterminé, d'une part, la teneur en carbone, et d'autre part, la radioactivité de chacune des sept fractions. Les tableaux II et III résument ces résultats dont se dégagent certaines conclusions.

En ce qui regarde la teneur en carbone, on ne constate, pour les diverses fractions, guère de modification importante entre le

TABLEAU II

Teneur en mg de carbone des diverses fractions humiques du sol, obtenues après décomposition de glucose radioactif

J	Fractions humiques totales mg C	% Carbone *					
		Acides fulviques	α-Humus	β-Humus	Acides hymatomélaniques	Humine hydrolysable	Humine non hydrolysable
7	147,4	12,9	19,6	8,5	8,1	12,5	38,4
15	173,6	16,1	21,1	6,2	7,9	13,0	35,7
30	126,2	14,8	22,6	6,3	8,1	16,0	32,2
45	124,4	18,4	23,2	9,8	7,1	11,5	30,0
60	127,4	15,2	23,4	8,5	7,6	15,3	30,0

* Les pourcentages sont exprimés par rapport à la somme totale du carbone contenu dans les 6 fractions humiques.

TABLEAU III

Radioactivité des 7 fractions organiques obtenues après décomposition du glucose radioactif *

J	Activité des substances solubles 10^3c/min	Activité totale des fractions humiques 10^3c/min	% Activité **					
			β-humus	Acides fulviques	α humus	Acides hymatomélaniques	Humine fraction hydrolysable	Humine fraction non hydrolysable
7	6,7	472,5	4,7	11,9	23,0	9,9	35,8	14,7
15	0,7	527,5	4,4	15,4	28,3	5,2	28,5	18,2
30	0,4	265,9	5,4	22,0	26,4	5,1	25,4	15,7
45	0,4	248,5	9,2	25,2	29,0	4,5	20,6	11,5
60	0,4	235,7	6,9	17,7	28,4	3,5	28,8	14,7

* Radioactivité initiale du substrat : 3,5 × 10^3 coups par minute (c/min)

** Les pourcentages sont exprimés en mille coups par minute par rapport à la radioactivité totale des 6 fractions humiques.

septième et le soixantième jour; pour l'humine non hydrolysable cependant, il passe de 38,9% à 30,0%. A cet égard, il ne faut pas perdre de vue que la fraction "humine" contient éventuellement aussi la cellulose et la lignine, dont la décomposition microbienne expliquerait la diminution de teneur en carbone de cette fraction.

La répartition du carbone organique dans le sol d'après le tableau II permet de classer par ordre d'importance décroissante, les diverses fractions de la façon suivante: humine non hydrolysable (30 à 39 %), α-humus (19 à 23%), ac. fulviques (13 à 18,4%), humine hydrolysable (11,5 à 15,3%), acides hymatomélaniques, β-humus et subst. solubles.

Pour ce qui concerne la radioactivité des diverses fractions, le tableau III montre tout d'abord que, déjà après 7 jours, *chacune des sept fractions* est radioactive. Cela implique notamment que déjà en 7 jours, on retrouve une partie du carbone radioactif sous forme de substances humiques.

On observe encore que, proportionnellement, la radioactivité des acides fulviques et celle de l'humus augmente avec le temps, tandis que la radioactivité des acides hymatomélaniques et celle de la fraction humine hydrolysable varie en sens inverse.

Enfin se dégage encore de ce tableau, que les fractions α-humus, acides fulviques et humine hydrolysable totalisent ensemble 70 à 75% de la radioactivité des fractions organiques.

RESUMÉ

Déjà après 7 jours on retrouve, sous forme de substances humiques, une partie du carbone radioactif provenant du glucose C^{14}. Les six fractions humiques du sol qui ont été étudiées sont radioactives dans ces conditions. Cependant, les fractions α-humus, acides fulviques et humine hydrolysable totalisent ensemble 70 à 75% de la radioactivité.

Reçu le 20 septembre 1957.

BIBLIOGRAPHIE

1 Bremner, J. M., Some observation on the oxidation of soil organic matter in the presence of alkali J. Soil sci. **1**, 198–204 (1950).

2 Simonart, P. et Mayaudon, J., Étude de la décomposition de la matière organique dans le sol au moyen de carbone radioactif. I. Cinétique de l'oxydation en CO_2 de divers substrats radioactifs. Plant and Soil **9**, 367–375 (1958).

3 Van Slyke D. D. and Folch, J., Manometric carbon determination. J. Biol. Chem. **136**, 509–541 (1940).

4 Waksman S. A., Humus, origin, chemical composition and importance in nature. Baillère, Tindall & Cox, 1936, London.

5 Wittich, W., Der heutige Stand unseres Wissens von Humus, J. D. Sauerlander's Verlag, 1952, Frankfurt am Main.

Plant and Soil IX, no 4 July 1958

ÉTUDE DE LA DÉCOMPOSITION DE LA MATIÈRE ORGANIQUE DANS LE SOL AU MOYEN DE CARBONE RADIOACTIF *

II. DÉCOMPOSITION DU GLUCOSE RADIOACTIF DANS LE SOL

B. RÉPARTITION DE LA RADIOACTIVITÉ DANS L' α-HUMUS

par PAUL SIMONART et JACQUES MAYAUDON

Centre de Microbiologie du Sol et de l'Eau, Université de Louvain, Héverlé, Belgique

I. INTRODUCTION

Il a été montré que la décomposition du glucose C^{14} dans le sol donne lieu à la formation de fractions humiques radioactives [2] et que 75% de la radioactivité se trouve dans l'α-humus, les acides fulviques et l'humines hydrolysable. Il s'indique donc d'étudier plus en détail la répartition de la radioactivité dans chacune de ces différentes fractions; ceci pourrait donner des indications quant à la formation de ces substances. L'objet de la présente note concerne l'étude de la radioactivité de la fraction α-humus.

II. MÉTHODES EXPÉRIMENTALES

Les méthodes de décomposition de matière organique dans le sol, de fractionnement de la matière organique du sol, de dosage de carbone et de mesure de radioactivité ont été décrites précédemment [2 3].

L'hydrolyse de la fraction α-humus s'effectue en atmosphère d'azote afin d'éviter les dangers d'oxydation. On opère de la façon

* Travail effectué sous les auspices de l'Institut pour l'Encouragement de la Recherche Scientifique dans l'Industrie et l'Agriculture (I.R.S.I.A.)

suivante: On place environ 50 mg d'humus et 10 ml HCl 3*N* dans le dispositif représenté par la figure I, dont le bulbe inferieur a une capacité de 20 ml, et dont le col est pourvu d'un réfrigérant. On relie le tube *a* à une bonbonne d'azote et le tube *b* à une pompe à vide.

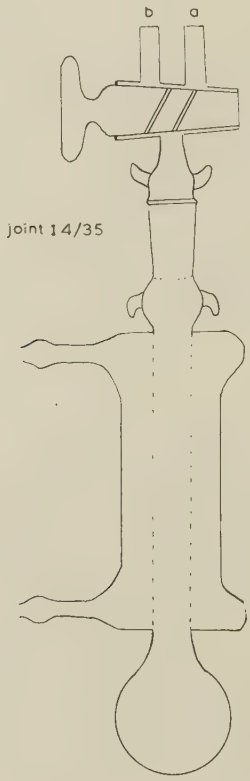


Figure 1. Tube à hydrolyse

Après évacuation de l'air contenu dans l'appareil, on y introduit de l'azote, que l'on évacue ensuite par la pompe à vide. On répète cette opération plusieurs fois, puis on ferme le robinet et maintient pendant 18 heures le bulbe du tube dans un bain de paraffine à 100°.

L'hydrolysat ainsi obtenu se partage en trois fractions:

- *la fraction soluble* (hS), de couleur jaune, qui renferme environ 50% du carbone contenu dans la fraction totale α-humus;
- *la fraction floculant à la neutralité* (hA), de couleur brun foncé, qui contient approximativement 15% du carbone de l'α-humus;

on sépare cette fraction de la fraction hS par centrifugation à pH = 7,0;

– *la fraction résiduelle* (hR), qui ne s'est pas solubilisée au cours de l'hydrolyse; elle est brun foncé, elle est insoluble dans les acides et contient environ 35% du carbone de l'α-humus.

III. RÉSULTATS ET DISCUSSION

L'expérience dont les résultats sont résumés au tableau I concerne la décomposition du glucose C^{14} dans le sol. La fraction α-humus isolée au cours d'une expérience précédente [2] a été hydrolysée selon les détails décrits ci-dessus. On a répété les examens après 7, 15, 30, 45 et 60 jours de décomposition.

TABLEAU I

Répartition du carbone et de la radioactivité dans les hydrolysats d'α-humus obtenu après décomposition de glucose radioactif

J	α-Humus		Fraction hS		Fraction hA		Fraction hR	
	mg C*	Act. tot.** 10^3 c/min	% C*	% Act.**	% C	% Act.	% C	% Act.
7	28,8	108,7	50,5	87,3	12,7	3,7	36,8	9,0
15	36,6	149.3	48,8	72,9	17,7	6,1	33,4	21,0
30	28,5	70,2	52,9	84,7	13,1	2,2	34,0	13,1
45	28,8	72,0	48,1	76,2	13,9	5,4	38,0	18,4
60	29,8	66,9	48,0	76,4	13,9	6,3	38,1	17,3

* La teneur en carbone est exprimée en mg; le pourcentage de carbone est exprimé par rapport à la radioactivité totale de la fraction α-humus.

** La radioactivité est exprimée en mille coups par minute (c/min), le pourcentage de radioactivité est exprimé par rapport à la radioactivité totale de la fraction α-humus.

Le tableau I fait ressortir que la radioactivité est sensiblement plus grande dans la fraction hS, où elle varie de 72,9% à 87,3%, que dans les deux fractions hA et hR. Ceci semble indiquer, d'une part, une synthèse plus rapide de la fraction hS que des fractions hA et hR, et, d'autre part, peut-être aussi une différence d'origine.

Il y a encore lieu de remarquer que 90% des substances radioactives de la première fraction sont fixés sur Dowex 50, dont on peut les éluer par NH_4OH 1,5*N*. Comme on sait que les acides aminés se comportent de la même façon et que Bremner [1] en a mis vingt-deux en évidence dans les hydrolysats d'humus, il serait donc intéressant d'établir avec certitude s'il s'agit là d'acides aminés radioactifs.

S'il se confirme que la radioactivité se trouve concentrée en majeure partie dans les acides aminés, il y aurait là une preuve certaine de l'intervention prépondérante du métabolisme quaternaire des micro-organismes du sol dans le phénomène d'humification.

IV. RESUMÉ

Le fractionnement d'un hydrolysat de la fraction α-humus radioactif formé en présence de glucose C^{14} montre que la plus grande partie de la radioactivité (72,9 à 87,3%) se retrouve dans la fraction soluble; quasi toute cette radioactivité est due à des substances fixées par le Dowex 50 et éluables par NH_4OH 1,5 *N*, ce qui est le cas des acides aminés.

Recu le 20 septembre 1957

BIBLIOGRAPHIE

1 Bremner, J. M., Nitrogen distribution and amino-acid composition of fractions of a humic acid from a chernozem soil. Z. Pflanzenernähr. Düng. Bodenk. **71**, 63 (1955).
2 Mayaudon, J. et Simonart, P., Étude de la décomposition de la matière organique dans le sol au moyen de carbone radioactif. II. Décomposition du glucose radioactif dans le sol A. Répartition de la radioactivité dans les fractions humiques du sol. Plant and Soil, **9**, 376–380 (1958).
3 Simonart, P. et Mayaudon J., Étude de la décomposition de la matière organique dans le sol au moyen de carbone radioactif. I. Cinétique de l'oxydation en CO_2 de divers substrats radioactifs. Plant and Soil **9**, 367-375 (1958).

Plant and Soil IX, no. 4 July 1958

EXPLORATORY POT EXPERIMENTS ON SENSITIVENESS OF DIFFERENT CROPS TO SODIUM: E. RED TABLE BEET

by J. M. WYBENGA and J. J. LEHR

Plant Nutrition Research Laboratory, Chilean Nitrate Agricultural Service, Wageningen, Netherlands

INTRODUCTION

It is well known that members of the Chenopodiaceae family including fodder beets, mangolds, sugar beets and spinach have a special preference for sodium. In this respect the red table beet is no exception. Although information on this crop is rather limited, it seems likely that sodium is an important nutrient for the red beet and that this element fulfills an essential function in this crop.

Harmer and Benne [2] found that on mucksoil, with ample supply of potassium, the yields of table beets increased considerably by adding sodium chloride. Furthermore an appreciable improvement of the appearance of the plants was obtained. With an application of 600 pounds per acre of a 0-8-24 mixture the leaves remained small and purplish, but with an extra addition of salt at the rate of 1,000 pounds per acre they developed healthy and green.

It should be observed that the treatments without addition of potassium but with administration of salt gave lower yields than the control plots, (*i.e.* with the 0 8 24 mixture). The authors therefore remark that "soda cannot perform all of the functions of potash in the metabolism of the plant" but in view of the positive results of salt in combination with potassium they conclude that for salt responsive crops, namely table beets, celery, mangolds, sugar beets, swiss chard and turnips, "the sodium ion appears nearly as much needed as a nutrient as is the potassium ion".

From their experiments with sodium chloride, sodium nitrate and sodium sulphate Sayre and Vittum [7] came to the conclusion

that table beets require sodium in addition to complete fertilizer and that sodium nitrate was the most effective form of sodium.

The importance of sodium for red beets appears furthermore from the exhaustive study of Pew [4] who summarized his results as follows: "When the minimum requirements of the plant for potassium are met then it appears that further application of sodium may be more efficient in increasing the yields than comparable amounts of potassium".

Sayre and Shafer [6] presented evidence showing that beets suffering from sodium deficiency as described by Harmer and Benne [2] promptly recovered to a healthy, normal green colour when supplied with this nutrient. In addition they obtained increased yields and improved plant vigour by adding sodium either in the nitrate or chloride form, even when ample potassium was present. Nitrogen without sodium failed to produce these responses.

In fertilization experiments on river clay [3] it appeared that the red beet derived more benefit from sodium than from potassium. Some investigators [5 8], who applied common salt attribute an important influence both to sodium and chlorine. Sayre and Vittum [7] on the other hand noticed that it is the sodium and not the chlorine that benefits the beets. Pew [4] when discussing the effect of common salt, concludes that "Strong, rather conclusive evidence has been presented which indicates that the sodium ion is of primary importance in creating the conditions more favourable for growth and development of the table beet". From other investigations [2 3 6] it also appears that, with table beet, the sodium effect is not specifically bound to the action of chlorine. In fact chlorine did not play an important part in the last-mentioned investigations, though it acted probably somewhat more favourably than sulphate.

EXPERIMENTAL

With red beets (var. Egyptian flat round) a pot trial was carried out on using a light humic sandy soil obtained from the site of our Laboratory.

Soil analysis

Organic matter, 2.0%; pH-water, 5.5; water-extractable P_2O_5, 5.5 mg per 100 grams of soil; P-citric 48; and extractable per 100 g soil: 10.6 mg K, 6.6 mg Na and 32 mg Ca.

The pots (c.m. 6 l) contained 7 kg soil. The moisture was brought up to

60 per cent of the waterholding capacity of the soil and fixed at this level during the experiments. The pots were sown with eight seeds per pot. The number of germinated plants was reduced to one per pot within a fortnight after sowing. All treatments were carried out sixfold.

Basal dressing

2 g $CaHPO_4.2\ H_2O$; 1.1 g $MgSO_4.7\ H_2O$; 200 mg borax; 100 mg $MnSO_4.4\ H_2O$; 50 mg $FeCl_3$ and 25 mg $CuSO_4.5\ H_2O$.

Nitrogen

2.5 g N in the form of Chilean nitrate of soda and of nitrate of lime respectively. One third was administered at the commencement of the experiment and the remainder in two equal portions as a topdressing.

Furthermore, treatments were inserted with two grades of Chilean potassic nitrate with 10 per cent and 14 per cent K_2O respectively.

Potassium

No potassium was applied to the treatments with the two grades of Chilean potassic nitrate which themselves contained 1300 and 1840 mg potassium per pot respectively. The other treatments received 0, 1145 or 3050 mg KCl per pot which corresponds with 0, 600 and 1800 mg potassium. Three fifths of these quantities were applied at the start of the experiment; the remainder was given as a topdressing.

RESULTS AND DISCUSSION

The development of the crop

The emergence and first development of the plants were normal. About two months after sowing (in the beginning of August) the plants of the treatments dressed with fertilizers containing sodium showed a better development of the leaves. Those with Chilean potassic nitrate had the most luxurious development.

The potassium levels exercised some influence on the leaf development in the Chilean nitrate treatments, as the growth of the plants without potash was slightly retarded. With nitrate of lime all treatments showed deficiency symptoms in the leaves (yellowing and scorching of the margins) even with the highest potash dose and although in the latter case the disease symptoms were less severe they can hardly be ascribed to potassium deficiency since the plants without potassium but dressed with Chilean nitrate were fully healthy. Therefore the disease rather appears to be due to sodium deficiency rather than to potassium deficiency.

The difference in physiological effect between sodium and pot-

assium was also reflected in the growth habit of the leaves. With a low supply of potassium they were narrow and tapered off towards the end, whereas with potassium a more broad-leaved development was obtained. Sodium appeared to influence the quality of the petiole which was longer, thicker and heavier, and contributed towards the healthy appearance of the leaves.

Crop yield and chemical composition

The present experiment confirms, in general, the findings of former investigations. Sodium improved substantially both the fresh weights and the dry-matter yields of table beet. The yield figures of the treatments with Chilean nitrate of soda were, in fact, of a quite different and higher level than those of the nitrate of lime treatments. Even at the highest potash dose which represents the most favourable case for nitrate of lime, sodium resulted in additional yields of 21 and 27% respectively for the fresh weight and 12 and 28% respectively, for the dry weight of beet roots and foliage (Table I, Figures 1 and 2).

TABLE I

Pot experiment with red table beets (1949)
Average yields of beets and leaves in g. per pot and dry-matter percentages

Treatments	Fresh weight		Dry-matter percentages	
	Leaves	Beets	Leaves	Beets
Chilean nitrate of soda				
0 mg K	99 ± 18	463 ± 15	14.8	10.3
600 mg K	120 ± 16	388 ± 21	16.3	11.5
1600 mg K	117 ± 9	375 ± 39[5]	12.1	13.0
Nitrate of lime				
0 mg K	50[5] ± 7	192 ± 22	22.0	13.5
600 mg K	52 ± 11	264 ± 20	17.4	13.6
1600 mg K	92 ± 8	300 ± 17	13.2	14.5
Chilean potash nitrate				
10%	126 ± 7	441 ± 28	11.2	12.0
14%	133 ± 17	441 ± 28	11.7	11.7

Potassium did not influence the results in the series with Chilean nitrate, as appears from the figures for total dry-matter yield. However, the low dose of potassium increased the production of foliage but depressed the development of beet roots. A positive effect of

potash dressing was observed in the series with nitrate of lime. The curve of the beet yields (Fig. 1) shows that potash has its maximum effect at the highest potash dose. The view of Sayre and Vittum [7] and others that sodium is essential for obtaining maximum yield of table beet has thus been confirmed by this investigation. It should certainly be considered that the dosage of sodium was much heavier than that of potassium (180 me Na in Chilean nitrate as against 40 me K at the highest level as potassium chloride). It is

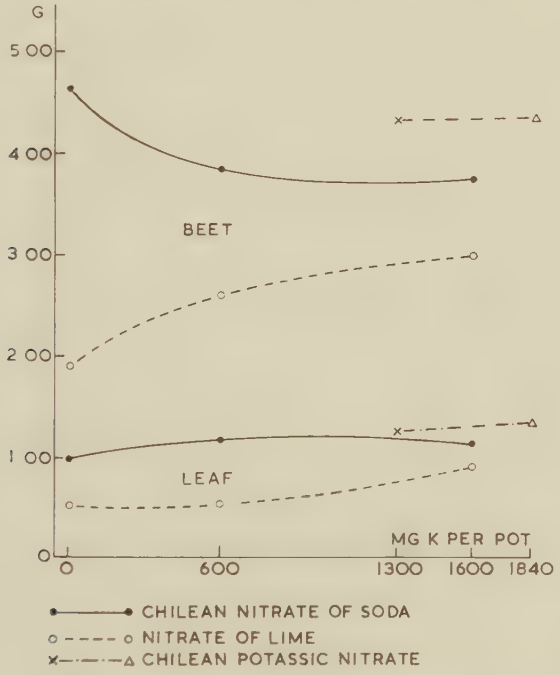


Fig. 1. Average fresh weight in g per pot

very probable, however, that a lower dosage of sodium would have sufficed for maximum production of beets since with Chilean potassic nitrate (containing either 10 or 14% K_2O and, therefore, a reduced amount of sodium) still higher yields were obtained than with nitrate of soda plus potassium chloride. Furthermore, the soil itself contained 31 me Na per pot which were utilised in the series with nitrate of lime but which masked to a certain extent the effect of the sodium added.

A firm relation between the sodium accumulation and the potash doses has only been found with the beet roots (Table II). This relation, a diminishing of the sodium accumulation with rising potassium doses, did not, however, include a constant sum of the sodium and potassium contents. From the analytical figures it

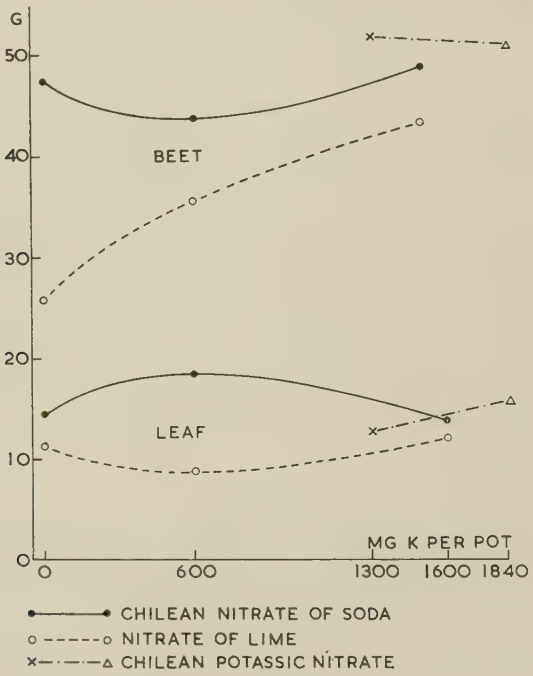


Fig. 2. Average dry-matter yield in g per pot

follows that potassium and sodium are not equivalent as nutrients for the beet plants. The potassium contents of the beet roots are higher than those of the leaves, whereas the contents of sodium and also those of calcium and magnesium, are highest in the leaves.

It is noteworthy that the addition of sodium increases the crude protein and phosphate contents of the beet roots whereas it exercises hardly any infleunce on the chlorine content. On the other hand, as has been also found with fodder beets [9], sodium nutrition decreases the phosphate content of the leaves.

In the series with nitrate of lime, potassium chloride tended to decrease the crude-protein content of the plants, but combined

with sodium nitrate it exercised practically no influence on this content. Sodium fertilization increased the total uptake of P_2O_5 (Table II) and induced an appreciable increase in the production of crude protein (Fig. 3).

TABLE II

Chemical analysis of red table beet

Treatments	% ash	me per 100 g dry matter				% of dry matter			total P_2O_5 in g	aqueous extract	
		K	Na	Ca	Mg	crude protein	P_2O_5	Cl		pH	red colour*
BEETS											
Chilean nitrate of soda											
0 mg K	11.5	22.2	130.6	10.9	6.2	21.4	1.5	0.1[5]	0.72	4.3	36.0
600 mg K	11.1	36.5	96.6	12.5	4.7	20.4	1.5	0.5	0.65	4.0	43.5
1600 mg K	10.1	53.6	70.9	12.0	4.3	20.7	1.3	0.7	0.65	5.0	28.0
Nitrate of lime											
0 mg K	8.6	38.0	16.9	37.6	7.8	16.9	1.3	0.1	0.34	4.0	41.5
600 mg K	7.6	54.7	11.1	22.8	6.4	13.1	1.2	0.4	0.43	4.1	38.0
1600 mg K	8.8	67.6	9.8	25.2	6.5	13.2	0.9	0.6	0.41	4.6	30.0
Chilean potash nitrate											
10% K_2O	9.2	43.6	62.0	21.8	6.5	16.6	1.3	0.1[5]	0.67	4.3	49.0
14% K_2O	9.9	56.4	60.7	11.6	5.1	18.4	1.3	0.1[5]	0.69	4.2	49.5
LEAVES											
Chilean nitrate of soda											
0 mg K	27.1	10.1	432.9	42.7	22.9	16.7	0.9	0.3	0.13	5.6	26.0
600 mg K	31.9	14.4	468.1	45.1	25.0	16.3	0.8	2.0	0.17	5.9	21.5
1600 mg K	29.3	34.3	363.9	57.5	23.3	16.2	0.8	2.3	0.11	5.6	22.0
Nitrate of lime											
0 mg K	19.5	20.1	73.5	209.1	55.2	19.6	1.7	0.6	0.19	5.8	25.0
600 mg K	23.4	27.7	88.2	252.0	67.4	16.1	1.6	1.5	0.14	5.3	23.5
1600 mg K	27.0	63.8	84.2	260.8	54.2	15.9	2.8	2.8	0.10	5.6	17.5
Chilean potash nitrate											
10% K_2O	26.8	21.7	415.6	58.1	25.8	19.6	0.8	0.4	0.10	5.7	26.0
14% K_2O	27.4	37.2	339.3	55.9	32.2	17.7	0.9	0.3	0.13	5.8	26.0

* % of light absorption with green filter (approx. 530 mμ)

Both the data available from the literature and the results of the experiment described leave no doubt that sodium is a very important nutrient for red beets. Some investigators, among whom Buchner[1], who specifically referred to the effect of common salt,

have questioned the importance of sodium as a plant nutrient and suggested that the effects obtained must be mainly ascribed to chlorine. Although this element has a certain value for the growth of beets, especially for the production of foliage, Buchner's statements certainly misrepresent the facts, the more so as they are based on a material which contains some peculiar inconsistencies. Apart from the arguments already mentioned in the introduction, reference can be made to the effect of sodium nitrate in the

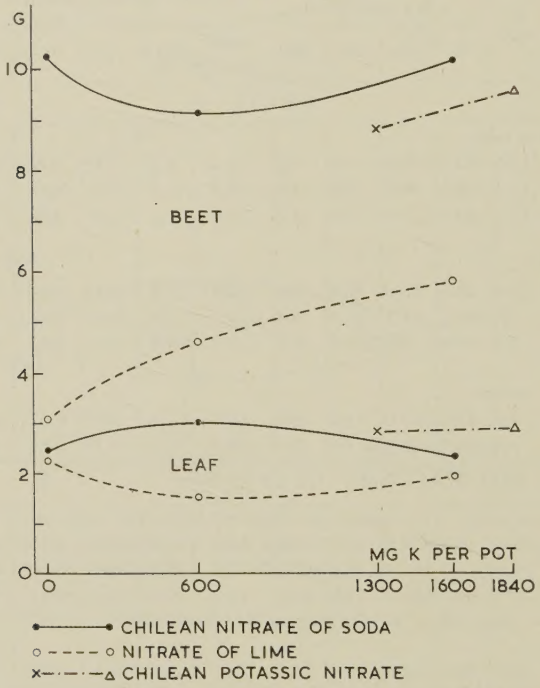


Fig. 3. Average yield of crude protein

present experiment and to the fact that Chilean potassic nitrate gave higher yields than the treatments with Chilean nitrate plus comparable amounts of potassium in the form of potassium chloride (Figs. 1 and 2). At least in the experiments in question chlorine did not have a special influence *.

* In the preceding paper of this series (J. J. Lehr and J. M. Wybenga, Exploratory pot experiments on sensitiveness of different crops to sodium: D. Barley, Plant and Soil, **9**, 3 (1958)) we suggested a combined effect of sodium and chlorine in barley. For other crops, *i.e.* spinach, oats, flax and table beet no special indications of such an effect would follow from the available data.

Chilean potassic nitrate, apart from producing the best yields, also improved the appearance of the plants and the colour of foliage and beet roots. This was of a deep red as could be shown by measuring the light absorption of aqueous extracts with a red filter.

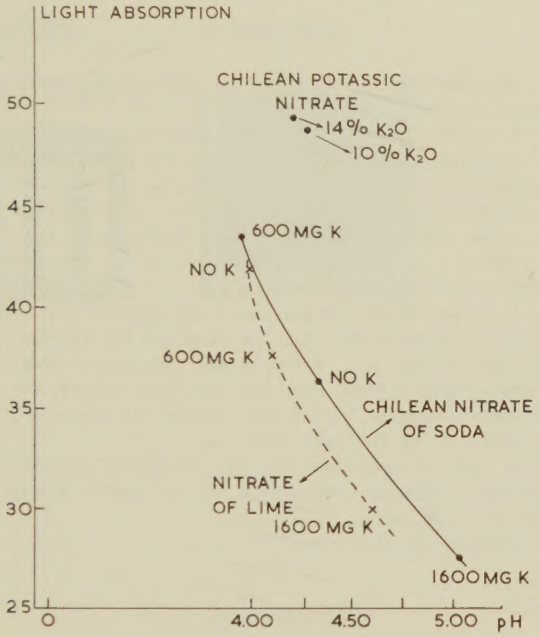


Fig. 4. Red colour of beets in relation to pH of extract

For the leaves no regular relation was found between pH and colour of the extracts, but a distinct relation between these factors and also a dependency on the kind of nitrate applied exists with the beets (Fig. 4).

SUMMARY

In pot experiments with red table beet, cultivated on a humic sandy soil, higher yields were obtained with sodium-containing N-fertilizers, *i.e.* Chilean nitrate of soda and particularly with Chilean potassic nitrate.

The results indicate that sodium is not only essential for maximum yields of this beet crop but also for good quality and healthy development. Beets fertilized with sodium nitrate were also characterized by a higher content of both crude protein and phosphate.

The beets from the Chilean Potassic nitrate treatments showed a deep red colour, which was more intensive the lower the pH of the aqueous extract of the dry matter of the beet roots.

Received November 18, 1957

LITERATURE

1 Buchner, A., Zur Wirkung von Natrium und Chlor bei der Rübendüngung. Z. für Acker- u. Pflanzenbau **93**, 523–528 (1951).
2 Harmer, P. M. and Benne, E. J., Effects of applying common salt to a muck soil on the yield, composition, and quality of certain vegetable crops and on the composition of the soil producing them. J. Am. Soc. Agron. **33**, 952–979 (1941).
3 Knoppien, P., Resultaten van bemestingsproeven op rivierklei bij enkele groenteteelt gewassen in de volle grond. Mededel. Direc. Tuinbouw **15**, 625–638 (1952).
4 Pew, W. D., Effects of the sodium and chlorine ions on the growth and yield of table beets. Thesis 1949, Cornell University.
5 Raleigh, G. J., Effects of the sodium and of the chloride ion in the nutrition of table beet in culture solutions. Proc. Am. Soc. Hort. Sci. **51**, 433–436 (1948).
6 Sayre, C. B. and Shafer, J. I., Effect of side dressings of different sodium and nitrogenous salts on yield of beets. Proc. Am. Soc. Hort. Sci. **44**, 453–456 (1944).
7 Sayre, C. B. and Vittum, M. T., Effect of sodium salts and rate of seeding on the yield of beets. Agron. J., **41**, 235–239 (1949).
8 Steenbjerg, F. and Dorph-Petersen, K., Kvaelstof, Kalium og Natrium til Bederoer (Nitrogen, potassium and sodium for red beets). Tidsskr. Planteavl **47**, 668–672 (1943) (C.A. **38**, 6465).
9 Unpublished results of our Laboratory.